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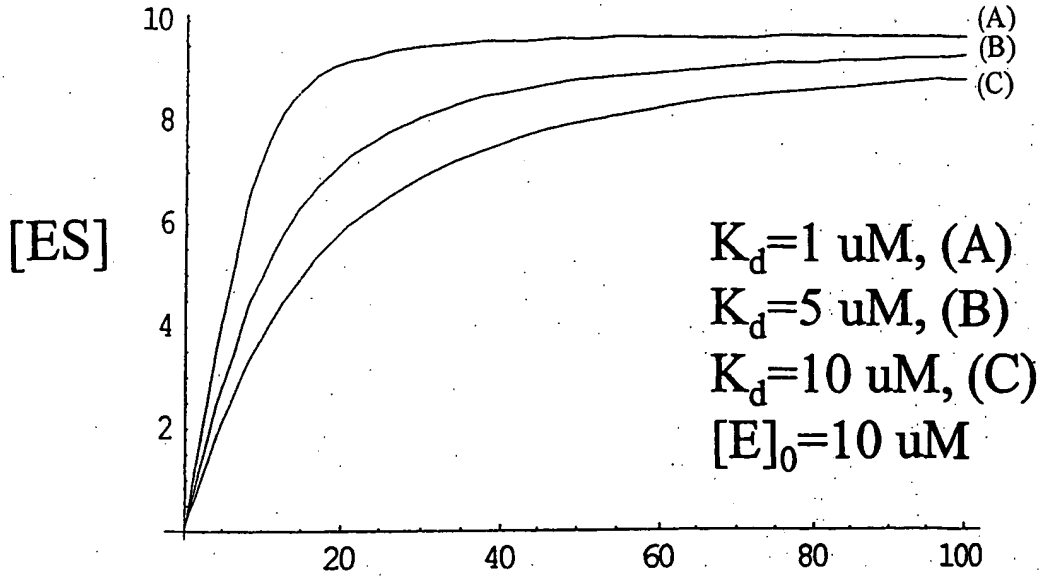
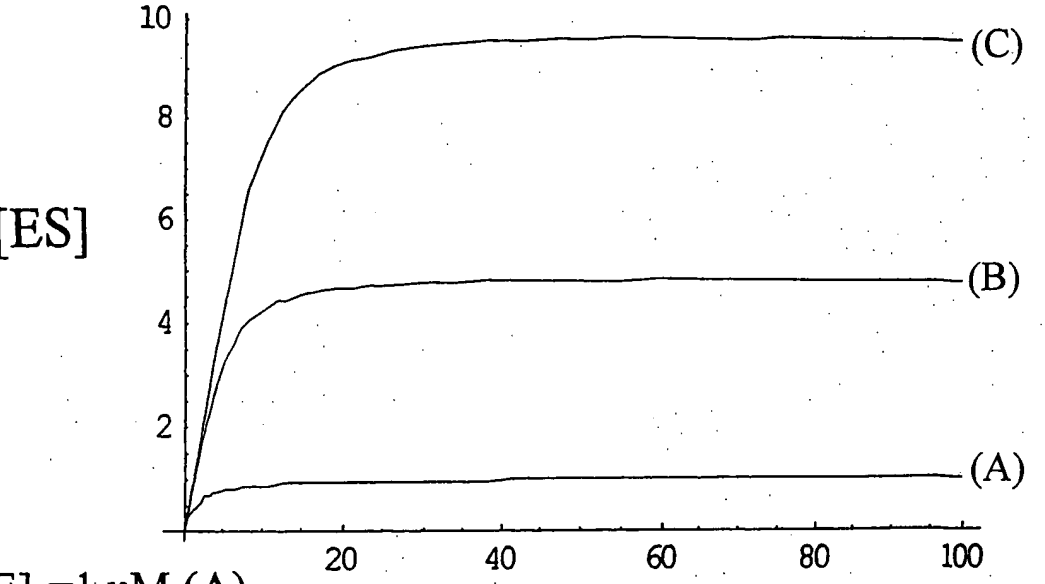



FIG. 1




$[E]_0$=1 uM (A)
$[E]_0$=5 uM (B)
$[E]_0$=10 uM (C)
$K_d$=1uM

FIG. 2

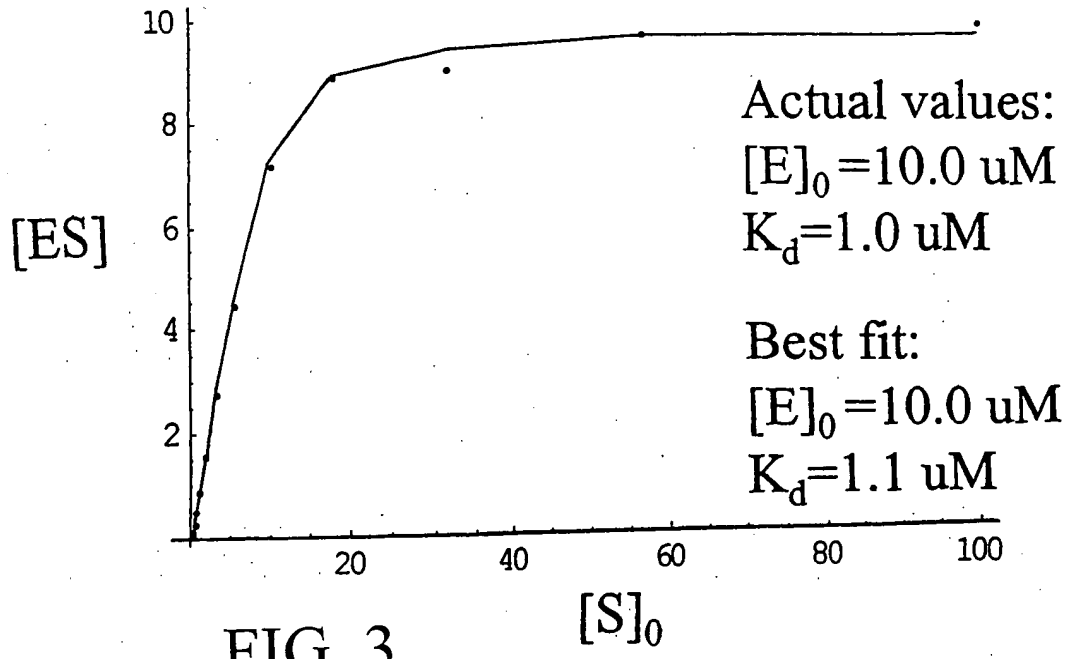



FIG. 3

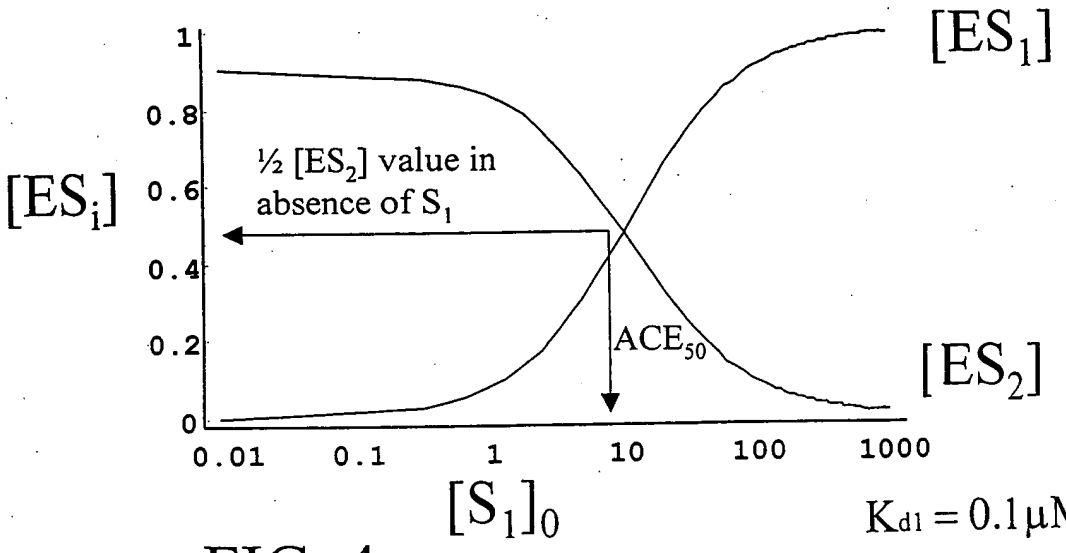



FIG. 4

$K_{d1} = 0.1 \mu M$

$K_{d2} = 1.0$

$[S_1]$ = variable

$[S_2]_0 = 10$

$[E]_0 = 1$

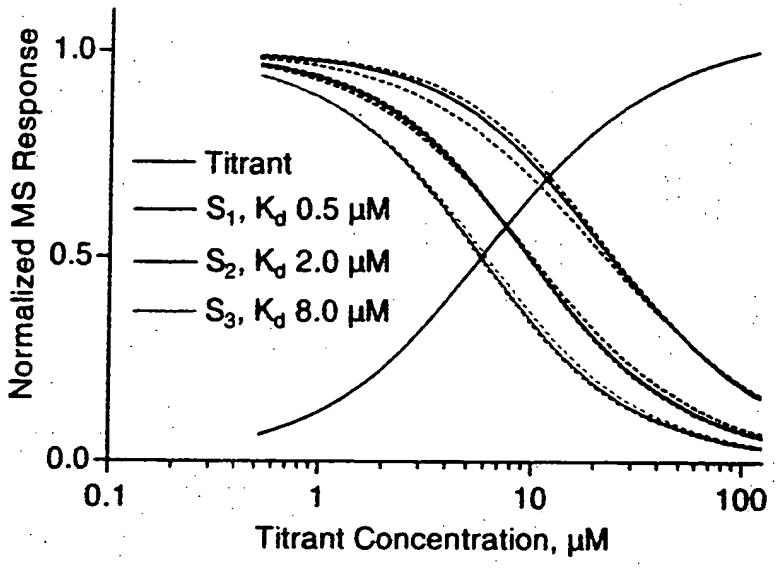

Normalized MS Response
1.0
0.5
0.0
0.1
1
10
100
Titrant Concentration, µM
Titrant
S1, Kd 0.5 µM
S2, Kd 2.0 µM
S3, Kd 8.0 µM

FIG. 5A

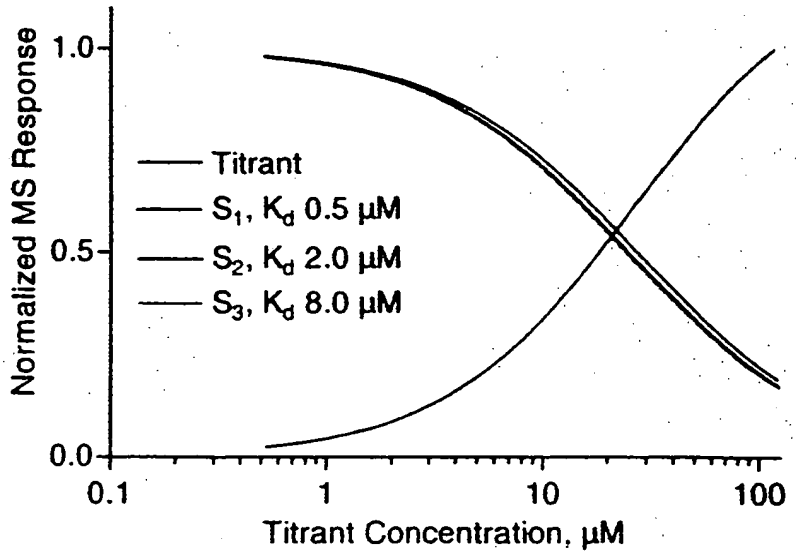

Normalized MS Response
1.0
0.5
0.0
0.1
1
10
100
Titrant Concentration, µM
Titrant
S1, Kd 0.5 µM
S2, Kd 2.0 µM
S3, Kd 8.0 µM

FIG. 5B

$$
\begin{aligned}
kd2 = & \\
& -\left(-4\,ace50^2 + 5\,ace50\,e0 - 2\,e0^2 + 2\,ace50\,kd1 - 4\,e0\,kd1 + 2\,kd1^2 + 4\,ace50\right. \\
& \left.\quad s20 - 2\,e0\,s20 - 6\,kd1\,s20\right) \Big/ \left(4\,(2\,ace50 - e0 - 2\,kd1)\right) + \\
& \frac{1}{2}\sqrt{\left(\left(-4\,ace50^2 + 5\,ace50\,e0 - 2\,e0^2 + 2\,ace50\,kd1 - 4\,e0\,kd1 + \right.\right.} \\
& \qquad \left.\left. 2\,kd1^2 + 4\,ace50\,s20 - 2\,e0\,s20 - 6\,kd1\,s20\right)^2 \Big/ \right. \\
& \qquad \left(4\,(2\,ace50 - e0 - 2\,kd1)^2\right) - \frac{1}{3\,(2\,ace50 - e0 - 2\,kd1)} \\
& \qquad \left(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + 6\,ace50\,e0\,kd1 - \right. \\
& \qquad\quad 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + 8\,ace50^2\,s20 - \\
& \qquad\quad 14\,ace50\,e0\,s20 + 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + \\
& \qquad\quad \left. 4\,kd1^2\,s20 + 4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\right) + \\
& \qquad \left(\left(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + \right.\right. \\
& \qquad\quad 10\,e0\,kd1^2 + 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
& \qquad\quad 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + 4\,ace50\,s20^2 - \\
& \qquad\quad \left.\left. 2\,e0\,s20^2 - 12\,kd1\,s20^2\right)^2 + 24\,(2\,ace50 - e0 - 2\,kd1)\right. \\
& \qquad\quad kd1^2\left(2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3\right) + \\
& \qquad\quad 12\,kd1\left(4\,ace50^2 - 5\,ace50\,e0 + 2\,e0^2 - 2\,ace50\,kd1 + \right. \\
& \qquad\quad\quad \left. 4\,e0\,kd1 - 2\,kd1^2 - 4\,ace50\,s20 + 2\,e0\,s20 + 6\,kd1\,s20\right) \\
& \qquad\quad \left(ace50\,e0^2 + 4\,e0^2\,kd1 - ace50\,e0\,s20 - 2\,e0^2\,s20 - 7\,e0\,kd1\right. \\
& \qquad\quad\quad \left.\left. s20 - 2\,ace50\,s20^2 + 5\,e0\,s20^2 - 2\,kd1\,s20^2 - 2\,s20^3\right)\right) \Big/ \\
& \left(3\,2^{2/3}\,(2\,ace50 - e0 - 2\,kd1)\left(2\left(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - \right.\right.\right. \\
& \qquad\quad 2\,e0^3 + 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + \\
& \qquad\quad 10\,e0\,kd1^2 + 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
& \qquad\quad 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + \\
& \qquad\quad \left.\left.\left. 4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\right)^3 + \right.\right.
\end{aligned}
$$

FIG. 6A

$$
\begin{aligned}
&108\,kd1^2\,(4\,ace50^2 - 5\,ace50\,e0 + 2\,e0^2 - 2\,ace50\,kd1 + \\
&\quad 4\,e0\,kd1 - 2\,kd1^2 - 4\,ace50\,s20 + 2\,e0\,s20 + \\
&\quad 6\,kd1\,s20)^2\,(2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - \\
&\quad 4\,s20^3) - 144\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2 \\
&\quad (-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + 6\,ace50\,e0\,kd1 - \\
&\quad 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + 8\,ace50^2\,s20 - 14\,ace50 \\
&\quad e0\,s20 + 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + \\
&\quad 4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2) \\
&\quad (2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3) + \\
&36\,kd1\,(4\,ace50^2 - 5\,ace50\,e0 + 2\,e0^2 - 2\,ace50\,kd1 + \\
&\quad 4\,e0\,kd1 - 2\,kd1^2 - 4\,ace50\,s20 + 2\,e0\,s20 + \\
&\quad 6\,kd1\,s20)\,(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + \\
&\quad 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + \\
&\quad 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + 5\,e0^2\,s20 + \\
&\quad 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + 4\,ace50\,s20^2 - \\
&\quad 2\,e0\,s20^2 - 12\,kd1\,s20^2)\,(ace50\,e0^2 + 4\,e0^2\,kd1 - \\
&\quad ace50\,e0\,s20 - 2\,e0^2\,s20 - 7\,e0\,kd1\,s20 - \\
&\quad 2\,ace50\,s20^2 + 5\,e0\,s20^2 - 2\,kd1\,s20^2 - 2\,s20^3) + \\
&216\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2 \\
&\quad (ace50\,e0^2 + 4\,e0^2\,kd1 - ace50\,e0\,s20 - \\
&\quad 2\,e0^2\,s20 - 7\,e0\,kd1\,s20 - 2\,ace50\,s20^2 + \\
&\quad 5\,e0\,s20^2 - 2\,kd1\,s20^2 - 2\,s20^3)^2 + \\
&\sqrt{(-4\,((-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + \\
&\quad 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + \\
&\quad 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
&\quad 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + 4 \\
&\quad ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2)^2 + \\
&\quad 24\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2
\end{aligned}
$$

FIG. 6B

$$
\begin{aligned}
&\left(2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3\right) + \\
&12\,kd1\left(4\,ace50^2 - 5\,ace50\,e0 + \right. \\
&\quad 2\,e0^2 - 2\,ace50\,kd1 + 4\,e0\,kd1 - 2\,kd1^2 - \\
&\quad \left. 4\,ace50\,s20 + 2\,e0\,s20 + 6\,kd1\,s20\right) \\
&\quad \left(ace50\,e0^2 + 4\,e0^2\,kd1 - ace50\,e0\,s20 - \right. \\
&\quad 2\,e0^2\,s20 - 7\,e0\,kd1\,s20 - 2\,ace50\,s20^2 + \\
&\quad \left. 5\,e0\,s20^2 - 2\,kd1\,s20^2 - 2\,s20^3\right)\Big)^3 + \\
&\left(2\left(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + \right.\right. \\
&\quad 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + \\
&\quad 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
&\quad 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + 4 \\
&\quad \left.\left. ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\right)^3 + \right. \\
&108\,kd1^2\left(4\,ace50^2 - 5\,ace50\,e0 + \right. \\
&\quad 2\,e0^2 - 2\,ace50\,kd1 + 4\,e0\,kd1 - 2\,kd1^2 - \\
&\quad \left. 4\,ace50\,s20 + 2\,e0\,s20 + 6\,kd1\,s20\right)^2 \\
&\quad \left(2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3\right) - \\
&144\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2 \\
&\quad \left(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + \right. \\
&\quad 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + \\
&\quad 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
&\quad 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + \\
&\quad \left. 4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\right) \\
&\quad \left(2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3\right) + \\
&36\,kd1\left(4\,ace50^2 - 5\,ace50\,e0 + \right. \\
&\quad 2\,e0^2 - 2\,ace50\,kd1 + 4\,e0\,kd1 - 2\,kd1^2 - \\
&\quad \left. 4\,ace50\,s20 + 2\,e0\,s20 + 6\,kd1\,s20\right) \\
&\quad \left(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + \right. \\
&\quad \left. 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + \right.
\end{aligned}
$$

FIG. 6C

$$
\begin{aligned}
&\quad 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
&\quad 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + \\
&\quad 4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\big) \\
&\big(ace50\,e0^2 + 4\,e0^2\,kd1 - ace50\,e0\,s20 - \\
&\quad 2\,e0^2\,s20 - 7\,e0\,kd1\,s20 - 2\,ace50\,s20^2 + \\
&\quad 5\,e0\,s20^2 - 2\,kd1\,s20^2 - 2\,s20^3\big) + \\
&216\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2 \\
&\big(ace50\,e0^2 + 4\,e0^2\,kd1 - ace50\,e0\,s20 - \\
&\quad 2\,e0^2\,s20 - 7\,e0\,kd1\,s20 - 2\,ace50\,s20^2 + \\
&\quad 5\,e0\,s20^2 - 2\,kd1\,s20^2 - 2\,s20^3\big)^2\big)^2\big)\big)^{1/3}\Big) + \\
&\frac{1}{6\,2^{1/3}\,(2\,ace50 - e0 - 2\,kd1)}\Big(\Big(2\big(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - \\
&\quad 2\,e0^3 + 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + \\
&\quad 10\,e0\,kd1^2 + 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
&\quad 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + \\
&\quad 4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\big)^3 + \\
&108\,kd1^2\big(4\,ace50^2 - 5\,ace50\,e0 + 2\,e0^2 - \\
&\quad 2\,ace50\,kd1 + 4\,e0\,kd1 - 2\,kd1^2 - \\
&\quad 4\,ace50\,s20 + 2\,e0\,s20 + 6\,kd1\,s20\big)^2 \\
&\big(2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3\big) - \\
&144\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2 \\
&\big(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + 6\,ace50\,e0 \\
&\quad kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + 8\,ace50^2\,s20 - 14 \\
&\quad ace50\,e0\,s20 + 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2 \\
&\quad s20 + 4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\big) \\
&\big(2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3\big) + \\
&36\,kd1\,\big(4\,ace50^2 - 5\,ace50\,e0 + 2\,e0^2 - 2\,ace50\,kd1 + 4\,e0
\end{aligned}
$$

FIG. 6D

$$
\begin{aligned}
&\text{kd1} - 2\,\text{kd1}^2 - 4\,\text{ace50}\,\text{s20} + 2\,\text{e0}\,\text{s20} + 6\,\text{kd1}\,\text{s20}\big) \\
&\big(-4\,\text{ace50}^2\,\text{e0} + 6\,\text{ace50}\,\text{e0}^2 - 2\,\text{e0}^3 + 6\,\text{ace50}\,\text{e0} \\
&\quad \text{kd1} - 4\,\text{e0}^2\,\text{kd1} + 10\,\text{e0}\,\text{kd1}^2 + 8\,\text{ace50}^2\,\text{s20} - 14 \\
&\quad \text{ace50}\,\text{e0}\,\text{s20} + 5\,\text{e0}^2\,\text{s20} + 2\,\text{e0}\,\text{kd1}\,\text{s20} + 4\,\text{kd1}^2 \\
&\quad \text{s20} + 4\,\text{ace50}\,\text{s20}^2 - 2\,\text{e0}\,\text{s20}^2 - 12\,\text{kd1}\,\text{s20}^2\big) \\
&\big(\text{ace50}\,\text{e0}^2 + 4\,\text{e0}^2\,\text{kd1} - \text{ace50}\,\text{e0}\,\text{s20} - 2\,\text{e0}^2 \\
&\quad \text{s20} - 7\,\text{e0}\,\text{kd1}\,\text{s20} - 2\,\text{ace50}\,\text{s20}^2 + 5 \\
&\quad \text{e0}\,\text{s20}^2 - 2\,\text{kd1}\,\text{s20}^2 - 2\,\text{s20}^3\big) + \\
&216\,(2\,\text{ace50} - \text{e0} - 2\,\text{kd1})\,\text{kd1}^2 \\
&\big(\text{ace50}\,\text{e0}^2 + 4\,\text{e0}^2\,\text{kd1} - \text{ace50}\,\text{e0}\,\text{s20} - \\
&\quad 2\,\text{e0}^2\,\text{s20} - 7\,\text{e0}\,\text{kd1}\,\text{s20} - 2\,\text{ace50}\,\text{s20}^2 + \\
&\quad 5\,\text{e0}\,\text{s20}^2 - 2\,\text{kd1}\,\text{s20}^2 - 2\,\text{s20}^3\big)^2 + \\
&\sqrt{\Big(-4\,\big((-4\,\text{ace50}^2\,\text{e0} + 6\,\text{ace50}\,\text{e0}^2 - 2\,\text{e0}^3 + \\
&\qquad 6\,\text{ace50}\,\text{e0}\,\text{kd1} - 4\,\text{e0}^2\,\text{kd1} + 10\,\text{e0}\,\text{kd1}^2 + \\
&\qquad 8\,\text{ace50}^2\,\text{s20} - 14\,\text{ace50}\,\text{e0}\,\text{s20} + \\
&\qquad 5\,\text{e0}^2\,\text{s20} + 2\,\text{e0}\,\text{kd1}\,\text{s20} + 4\,\text{kd1}^2\,\text{s20} + 4 \\
&\qquad \text{ace50}\,\text{s20}^2 - 2\,\text{e0}\,\text{s20}^2 - 12\,\text{kd1}\,\text{s20}^2\big)^2 + \\
&\quad 24\,(2\,\text{ace50} - \text{e0} - 2\,\text{kd1})\,\text{kd1}^2 \\
&\quad \big(2\,\text{e0}^3 - 9\,\text{e0}^2\,\text{s20} + 12\,\text{e0}\,\text{s20}^2 - 4\,\text{s20}^3\big) + \\
&\quad 12\,\text{kd1}\,\big(4\,\text{ace50}^2 - 5\,\text{ace50}\,\text{e0} + \\
&\qquad 2\,\text{e0}^2 - 2\,\text{ace50}\,\text{kd1} + 4\,\text{e0}\,\text{kd1} - 2\,\text{kd1}^2 - \\
&\qquad 4\,\text{ace50}\,\text{s20} + 2\,\text{e0}\,\text{s20} + 6\,\text{kd1}\,\text{s20}\big) \\
&\quad \big(\text{ace50}\,\text{e0}^2 + 4\,\text{e0}^2\,\text{kd1} - \text{ace50}\,\text{e0}\,\text{s20} - \\
&\qquad 2\,\text{e0}^2\,\text{s20} - 7\,\text{e0}\,\text{kd1}\,\text{s20} - 2\,\text{ace50}\,\text{s20}^2 + \\
&\qquad 5\,\text{e0}\,\text{s20}^2 - 2\,\text{kd1}\,\text{s20}^2 - 2\,\text{s20}^3\big)\Big)^3 + \\
&\big(2\,\big(-4\,\text{ace50}^2\,\text{e0} + 6\,\text{ace50}\,\text{e0}^2 - 2\,\text{e0}^3 + \\
&\qquad 6\,\text{ace50}\,\text{e0}\,\text{kd1} - 4\,\text{e0}^2\,\text{kd1} + 10\,\text{e0}\,\text{kd1}^2 +
\end{aligned}
$$

FIG. 6E

$$
\begin{aligned}
&8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
&5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + 4 \\
&ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\big)^3 + \\
&108\,kd1^2\big(4\,ace50^2 - 5\,ace50\,e0 + \\
&2\,e0^2 - 2\,ace50\,kd1 + 4\,e0\,kd1 - 2\,kd1^2 - \\
&4\,ace50\,s20 + 2\,e0\,s20 + 6\,kd1\,s20\big)^2 \\
&\big(2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3\big) - \\
&144\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2 \\
&\big(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + \\
&6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + \\
&8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
&5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + \\
&4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\big) \\
&\big(2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3\big) + \\
&36\,kd1\big(4\,ace50^2 - 5\,ace50\,e0 + 2\,e0^2 - \\
&2\,ace50\,kd1 + 4\,e0\,kd1 - 2\,kd1^2 - \\
&4\,ace50\,s20 + 2\,e0\,s20 + 6\,kd1\,s20\big) \\
&\big(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + \\
&6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + \\
&8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
&5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + \\
&4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\big) \\
&\big(ace50\,e0^2 + 4\,e0^2\,kd1 - ace50\,e0\,s20 - \\
&2\,e0^2\,s20 - 7\,e0\,kd1\,s20 - 2\,ace50\,s20^2 + \\
&5\,e0\,s20^2 - 2\,kd1\,s20^2 - 2\,s20^3\big) + \\
&216\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2 \\
&\big(ace50\,e0^2 + 4\,e0^2\,kd1 - ace50\,e0\,s20 - 2 \\
&e0^2\,s20 - 7\,e0\,kd1\,s20 - 2\,ace50\,s20^2 + 5
\end{aligned}
$$

FIG. 6F

$$e0\,s20^2 - 2\,kd1\,s20^2 - 2\,s20^3)^2)^2))^{1/3}))) -$$

$$\frac{1}{2}\sqrt{\Big((-4\,ace50^2 + 5\,ace50\,e0 - 2\,e0^2 + 2\,ace50\,kd1 -$$

$$4\,e0\,kd1 +$$
$$2\,kd1^2 +$$
$$4\,ace50$$
$$s20 - 2$$
$$e0\,s20 - 6$$
$$kd1\,s20)^2 \Big/$$
$$\big(2\,(2\,ace50 - e0 - 2\,kd1)^2\big) -$$
$$\frac{1}{3\,(2\,ace50 - e0 - 2\,kd1)}$$
$$\big(2$$
$$\big(-4\,ace50^2\,e0 +$$
$$6\,ace50\,e0^2 -$$
$$2\,e0^3 +$$
$$6\,ace50\,e0\,kd1 -$$
$$4\,e0^2\,kd1 +$$
$$10\,e0\,kd1^2 +$$
$$8\,ace50^2\,s20 -$$
$$14\,ace50\,e0\,s20 +$$
$$5\,e0^2\,s20 +$$
$$2\,e0\,kd1\,s20 +$$
$$4\,kd1^2\,s20 +$$
$$4\,ace50\,s20^2 -$$
$$2\,e0\,s20^2 -$$
$$12\,kd1\,s20^2)) -$$
$$\Big((-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + 6\,ace50\,e0\,kd1 -$$
$$4\,e0^2\,kd1 + 10\,e0\,kd1^2 +$$

FIG. 6G

$$
\begin{aligned}
&-8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
&\quad 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + \\
&\quad 4\,kd1^2\,s20 + 4\,ace50\,s20^2 - \\
&\quad 2\,e0\,s20^2 - 12\,kd1\,s20^2\Big)^2 + \\
&24\,(2\,ace50 - e0 - 2\,kd1) \\
&\quad kd1^2 \\
&\quad \left(2\,e0^3 - 9\,e0^2\,s20 + \right. \\
&\qquad \left. 12\,e0\,s20^2 - 4\,s20^3\right) + \\
&12\,kd1\,\left(4\,ace50^2 - 5\,ace50\,e0 + 2\,e0^2 - \right. \\
&\qquad 2\,ace50\,kd1 + 4\,e0\,kd1 - \\
&\qquad 2\,kd1^2 - 4\,ace50\,s20 + \\
&\qquad \left. 2\,e0\,s20 + 6\,kd1\,s20\right) \\
&\quad \left(ace50\,e0^2 + 4\,e0^2\,kd1 - ace50\,e0\,s20 - \right. \\
&\qquad 2\,e0^2\,s20 - 7\,e0\,kd1\,s20 - \\
&\qquad 2\,ace50\,s20^2 + 5\,e0\,s20^2 - \\
&\qquad \left. 2\,kd1\,s20^2 - 2\,s20^3\right)\Big)\Big/ \\
&\left(3\,2^{2/3}\,(2\,ace50 - e0 - 2\,kd1)\right. \\
&\quad \Big(2\,\big(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + 6\,ace50\,e0\,kd1 - \\
&\qquad 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + 8\,ace50^2\,s20 - \\
&\qquad 14\,ace50\,e0\,s20 + 5\,e0^2\,s20 + \\
&\qquad 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + \\
&\qquad 4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\big)^3 + \\
&\quad 108\,kd1^2\,\left(4\,ace50^2 - 5\,ace50\,e0 + 2\,e0^2 - \right. \\
&\qquad 2\,ace50\,kd1 + 4\,e0\,kd1 - 2\,kd1^2 - \\
&\qquad \left. 4\,ace50\,s20 + 2\,e0\,s20 + 6\,kd1\,s20\right)^2 \\
&\quad \left(2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3\right) -
\end{aligned}
$$

FIG. 6H

$$
\begin{aligned}
&144\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2\,(-4\,ace50^2\,e0 + \\
&\quad 6\,ace50\,e0^2 - 2\,e0^3 + 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + \\
&\quad 10\,e0\,kd1^2 + 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
&\quad 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + \\
&\quad 4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2) \\
&\quad (2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3) + \\
&36\,kd1\,(4\,ace50^2 - 5\,ace50\,e0 + 2\,e0^2 - \\
&\quad 2\,ace50\,kd1 + 4\,e0\,kd1 - 2\,kd1^2 - \\
&\quad 4\,ace50\,s20 + 2\,e0\,s20 + 6\,kd1\,s20) \\
&\quad (-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + \\
&\quad 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + \\
&\quad 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
&\quad 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + \\
&\quad 4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2) \\
&\quad (ace50\,e0^2 + 4\,e0^2\,kd1 - ace50\,e0\,s20 - \\
&\quad 2\,e0^2\,s20 - 7\,e0\,kd1\,s20 - 2\,ace50\,s20^2 + \\
&\quad 5\,e0\,s20^2 - 2\,kd1\,s20^2 - 2\,s20^3) + \\
&216\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2 \\
&\quad (ace50\,e0^2 + 4\,e0^2\,kd1 - ace50\,e0\,s20 - \\
&\quad 2\,e0^2\,s20 - 7\,e0\,kd1\,s20 - 2\,ace50\,s20^2 + \\
&\quad 5\,e0\,s20^2 - 2\,kd1\,s20^2 - 2\,s20^3)^2 + \\
&\sqrt{(-4\,((-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + \\
&\quad 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + \\
&\quad 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
&\quad 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + 4 \\
&\quad ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2)^2 + \\
&\quad 24\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2 \\
&\quad (2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3) +
\end{aligned}
$$

FIG. 6I

$$
\begin{aligned}
&12\,kd1\,(4\,ace50^2 - 5\,ace50\,e0 + \\
&\quad 2\,e0^2 - 2\,ace50\,kd1 + 4\,e0\,kd1 - 2\,kd1^2 - \\
&\quad 4\,ace50\,s20 + 2\,e0\,s20 + 6\,kd1\,s20) \\
&\quad (ace50\,e0^2 + 4\,e0^2\,kd1 - ace50\,e0\,s20 - \\
&\quad 2\,e0^2\,s20 - 7\,e0\,kd1\,s20 - 2\,ace50\,s20^2 + \\
&\quad 5\,e0\,s20^2 - 2\,kd1\,s20^2 - 2\,s20^3))^3 + \\
&(2\,(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + \\
&\quad 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + \\
&\quad 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
&\quad 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + 4 \\
&\quad ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2)^3 + \\
&108\,kd1^2\,(4\,ace50^2 - 5\,ace50\,e0 + \\
&\quad 2\,e0^2 - 2\,ace50\,kd1 + 4\,e0\,kd1 - 2\,kd1^2 - \\
&\quad 4\,ace50\,s20 + 2\,e0\,s20 + 6\,kd1\,s20)^2 \\
&\quad (2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3) - \\
&144\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2 \\
&\quad (-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + \\
&\quad 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + \\
&\quad 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
&\quad 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + \\
&\quad 4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2) \\
&\quad (2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3) + \\
&36\,kd1\,(4\,ace50^2 - 5\,ace50\,e0 + \\
&\quad 2\,e0^2 - 2\,ace50\,kd1 + 4\,e0\,kd1 - 2\,kd1^2 - \\
&\quad 4\,ace50\,s20 + 2\,e0\,s20 + 6\,kd1\,s20) \\
&\quad (-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + \\
&\quad 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + \\
&\quad 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 +
\end{aligned}
$$

FIG. 6J

$$5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + 4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\Big)\Big(ace50\,e0^2 + 4\,e0^2\,kd1 - ace50\,e0\,s20 - 2\,e0^2\,s20 - 7\,e0\,kd1\,s20 - 2\,ace50\,s20^2 + 5\,e0\,s20^2 - 2\,kd1\,s20^2 - 2\,s20^3\Big) + 216\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2\Big(ace50\,e0^2 + 4\,e0^2\,kd1 - ace50\,e0\,s20 - 2\,e0^2\,s20 - 7\,e0\,kd1\,s20 - 2\,ace50\,s20^2 + 5\,e0\,s20^2 - 2\,kd1\,s20^2 - 2\,s20^3\Big)^2\Big)^2\Big)\Big)^{1/3}\Bigg) -$$

$$\frac{1}{6\,2^{1/3}\,(2\,ace50 - e0 - 2\,kd1)}\Bigg(\Big(2\Big(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + 4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\Big)^3 + 108\,kd1^2\Big(4\,ace50^2 - 5\,ace50\,e0 + 2\,e0^2 - 2\,ace50\,kd1 + 4\,e0\,kd1 - 2\,kd1^2 - 4\,ace50\,s20 + 2\,e0\,s20 + 6\,kd1\,s20\Big)^2\Big(2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3\Big) - 144\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2\Big(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + 4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\Big)\Big(2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3\Big) + 36\,kd1\,\Big(4\,ace50^2 - 5\,ace50\,e0 + 2\,e0^2 - 2$$

FIG. 6K

$$
\begin{aligned}
&\text{ace50 kd1} + 4\,\text{e0 kd1} - 2\,\text{kd1}^2 - 4 \\
&\text{ace50 s20} + 2\,\text{e0 s20} + 6\,\text{kd1 s20}\big) \\
&\big(-4\,\text{ace50}^2\,\text{e0} + 6\,\text{ace50 e0}^2 - 2\,\text{e0}^3 + 6\,\text{ace50} \\
&\text{e0 kd1} - 4\,\text{e0}^2\,\text{kd1} + 10\,\text{e0 kd1}^2 + 8 \\
&\text{ace50}^2\,\text{s20} - 14\,\text{ace50 e0 s20} + 5\,\text{e0}^2 \\
&\text{s20} + 2\,\text{e0 kd1 s20} + 4\,\text{kd1}^2\,\text{s20} + 4 \\
&\text{ace50 s20}^2 - 2\,\text{e0 s20}^2 - 12\,\text{kd1 s20}^2\big) \\
&\big(\text{ace50 e0}^2 + 4\,\text{e0}^2\,\text{kd1} - \text{ace50 e0 s20} - 2\,\text{e0}^2 \\
&\text{s20} - 7\,\text{e0 kd1 s20} - 2\,\text{ace50 s20}^2 + 5 \\
&\text{e0 s20}^2 - 2\,\text{kd1 s20}^2 - 2\,\text{s20}^3\big) + \\
&216\,(2\,\text{ace50} - \text{e0} - 2\,\text{kd1})\,\text{kd1}^2 \\
&\big(\text{ace50 e0}^2 + 4\,\text{e0}^2\,\text{kd1} - \text{ace50 e0 s20} - \\
&2\,\text{e0}^2\,\text{s20} - 7\,\text{e0 kd1 s20} - 2\,\text{ace50 s20}^2 + \\
&5\,\text{e0 s20}^2 - 2\,\text{kd1 s20}^2 - 2\,\text{s20}^3\big)^2 + \\
&\sqrt{\Big(-4\,\Big(\big(-4\,\text{ace50}^2\,\text{e0} + 6\,\text{ace50 e0}^2 - 2\,\text{e0}^3 + \\
&6\,\text{ace50 e0 kd1} - 4\,\text{e0}^2\,\text{kd1} + 10\,\text{e0 kd1}^2 + \\
&8\,\text{ace50}^2\,\text{s20} - 14\,\text{ace50 e0 s20} + \\
&5\,\text{e0}^2\,\text{s20} + 2\,\text{e0 kd1 s20} + 4\,\text{kd1}^2\,\text{s20} + 4 \\
&\text{ace50 s20}^2 - 2\,\text{e0 s20}^2 - 12\,\text{kd1 s20}^2\big)^2 + \\
&24\,(2\,\text{ace50} - \text{e0} - 2\,\text{kd1})\,\text{kd1}^2 \\
&\big(2\,\text{e0}^3 - 9\,\text{e0}^2\,\text{s20} + 12\,\text{e0 s20}^2 - 4\,\text{s20}^3\big) + \\
&12\,\text{kd1}\,\big(4\,\text{ace50}^2 - 5\,\text{ace50 e0} + \\
&2\,\text{e0}^2 - 2\,\text{ace50 kd1} + 4\,\text{e0 kd1} - 2\,\text{kd1}^2 - \\
&4\,\text{ace50 s20} + 2\,\text{e0 s20} + 6\,\text{kd1 s20}\big) \\
&\big(\text{ace50 e0}^2 + 4\,\text{e0}^2\,\text{kd1} - \text{ace50 e0 s20} - \\
&2\,\text{e0}^2\,\text{s20} - 7\,\text{e0 kd1 s20} - 2\,\text{ace50 s20}^2 + \\
&5\,\text{e0 s20}^2 - 2\,\text{kd1 s20}^2 - 2\,\text{s20}^3\big)\Big)^3 +
\end{aligned}
$$

FIG. 6L

$$
\begin{aligned}
&\Big(2\big(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + \\
&\qquad 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + \\
&\qquad 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
&\qquad 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + 4 \\
&\qquad ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\big)^3 + \\
&108\,kd1^2\big(4\,ace50^2 - 5\,ace50\,e0 + \\
&\qquad 2\,e0^2 - 2\,ace50\,kd1 + 4\,e0\,kd1 - 2\,kd1^2 - \\
&\qquad 4\,ace50\,s20 + 2\,e0\,s20 + 6\,kd1\,s20\big)^2 \\
&\big(2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3\big) - \\
&144\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2 \\
&\big(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + \\
&\qquad 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + \\
&\qquad 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
&\qquad 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + \\
&\qquad 4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\big) \\
&\big(2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3\big) + \\
&36\,kd1\big(4\,ace50^2 - 5\,ace50\,e0 + 2\,e0^2 - \\
&\qquad 2\,ace50\,kd1 + 4\,e0\,kd1 - 2\,kd1^2 - \\
&\qquad 4\,ace50\,s20 + 2\,e0\,s20 + 6\,kd1\,s20\big) \\
&\big(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + \\
&\qquad 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + \\
&\qquad 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
&\qquad 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + \\
&\qquad 4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\big) \\
&\big(ace50\,e0^2 + 4\,e0^2\,kd1 - ace50\,e0\,s20 - \\
&\qquad 2\,e0^2\,s20 - 7\,e0\,kd1\,s20 - 2\,ace50\,s20^2 + \\
&\qquad 5\,e0\,s20^2 - 2\,kd1\,s20^2 - 2\,s20^3\big) + \\
&216\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2
\end{aligned}
$$

FIG. 6M

$$-\left(\text{ace50}\,\text{e0}^2 + 4\,\text{e0}^2\,\text{kd1} - \text{ace50}\,\text{e0}\,\text{s20} - 2\,\text{e0}^2\,\text{s20} - 7\,\text{e0}\,\text{kd1}\,\text{s20} - 2\,\text{ace50}\,\text{s20}^2 + 5\,\text{e0}\,\text{s20}^2 - 2\,\text{kd1}\,\text{s20}^2 - 2\,\text{s20}^3\right)^2\Big)^2\Big)\Big)^{1/3}\Bigg) +$$

$$\Bigg(-\left(-4\,\text{ace50}^2 + 5\,\text{ace50}\,\text{e0} - 2\,\text{e0}^2 + 2\,\text{ace50}\,\text{kd1} - 4\,\text{e0}\,\text{kd1} + 2\,\text{kd1}^2 + 4\,\text{ace50}\,\text{s20} - 2\,\text{e0}\,\text{s20} - 6\,\text{kd1}\,\text{s20}\right)^3 \Big/$$

$$(2\,\text{ace50} - \text{e0} - 2\,\text{kd1})^3 + \frac{1}{(2\,\text{ace50} - \text{e0} - 2\,\text{kd1})^2}$$

$$\Big(2\,\Big(-4\,\text{ace50}^2 + 5\,\text{ace50}\,\text{e0} - 2\,\text{e0}^2 + 2\,\text{ace50}\,\text{kd1} - 4\,\text{e0}\,\text{kd1} + 2\,\text{kd1}^2 + 4\,\text{ace50}\,\text{s20} - 2\,\text{e0}\,\text{s20} - 6\,\text{kd1}\,\text{s20}\Big)$$

$$\Big(-4\,\text{ace50}^2\,\text{e0} + 6\,\text{ace50}\,\text{e0}^2 - 2\,\text{e0}^3 + 6\,\text{ace50}\,\text{e0}\,\text{kd1} - 4\,\text{e0}^2\,\text{kd1} + 10\,\text{e0}\,\text{kd1}^2 + 8\,\text{ace50}^2\,\text{s20} - 14\,\text{ace50}\,\text{e0}\,\text{s20} + 5\,\text{e0}^2\,\text{s20} + 2\,\text{e0}\,\text{kd1}\,\text{s20} + 4\,\text{kd1}^2\,\text{s20} + 4\,\text{ace50}\,\text{s20}^2 - 2\,\text{e0}\,\text{s20}^2 - 12\,\text{kd1}\,\text{s20}^2\Big)\Big) -$$

$$\Big(8\,\text{kd1}\,\Big(\text{ace50}\,\text{e0}^2 + 4\,\text{e0}^2\,\text{kd1} - \text{ace50}\,\text{e0}\,\text{s20} - 2\,\text{e0}^2\,\text{s20} - 7\,\text{e0}\,\text{kd1}\,\text{s20} - 2\,\text{ace50}\,\text{s20}^2 + 5\,\text{e0}\,\text{s20}^2 - 2\,\text{kd1}\,\text{s20}^2 - 2\,\text{s20}^3\Big)\Big) \Big/$$

$$(2\,\text{ace50} - \text{e0} - 2\,\text{kd1})\Big) \Big/$$

$$\Bigg(4\,\sqrt{\Bigg(\left(-4\,\text{ace50}^2 + 5\,\text{ace50}\,\text{e0} - 2\,\text{e0}^2 + 2\,\text{ace50}\,\text{kd1} - 4\,\text{e0}\,\text{kd1} + 2\,\text{kd1}^2 + 4\,\text{ace50}\,\text{s20} - 2\,\text{e0}\,\text{s20} - 6\,\text{kd1}\,\text{s20}\right)^2 \Big/$$

$$\left(4\,(2\,\text{ace50} - \text{e0} - 2\,\text{kd1})^2\right) -$$

FIG. 6N

$$\frac{1}{3\,(2\,ace50 - e0 - 2\,kd1)}$$
$$\Big(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + 4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\Big) +$$
$$\Big(\big(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + 4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\big)^2 + 24\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2\,\big(2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3\big) + 12\,kd1\,\big(4\,ace50^2 - 5\,ace50\,e0 + 2\,e0^2 - 2\,ace50\,kd1 + 4\,e0\,kd1 - 2\,kd1^2 - 4\,ace50\,s20 + 2\,e0\,s20 + 6\,kd1\,s20\big)\,\big(ace50\,e0^2 + 4\,e0^2\,kd1 - ace50\,e0\,s20 - 2\,e0^2\,s20 - 7\,e0\,kd1\,s20 - 2\,ace50\,s20^2 + 5\,e0\,s20^2 - 2\,kd1\,s20^2 - 2\,s20^3\big)\Big)\Big/$$
$$\Big(3\,2^{2/3}\,(2\,ace50 - e0 - 2\,kd1)$$
$$\Big(2\,\big(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + 4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\big)^3 + 108\,kd1^2\,\big(4\,ace50^2 - 5\,ace50\,e0 +$$

FIG. 6O

$$
\begin{aligned}
&2\,e0^2 - 2\,ace50\,kd1 + 4\,e0\,kd1 - 2\,kd1^2 - \\
&4\,ace50\,s20 + 2\,e0\,s20 + 6\,kd1\,s20)^2 \\
&(2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3) - \\
&144\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2 \\
&(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + \\
&6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + \\
&8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
&5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + \\
&4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2) \\
&(2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3) + \\
&36\,kd1\,(4\,ace50^2 - 5\,ace50\,e0 + 2\,e0^2 - \\
&2\,ace50\,kd1 + 4\,e0\,kd1 - 2\,kd1^2 - \\
&4\,ace50\,s20 + 2\,e0\,s20 + 6\,kd1\,s20) \\
&(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + \\
&6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + \\
&8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
&5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + \\
&4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2) \\
&(ace50\,e0^2 + 4\,e0^2\,kd1 - ace50\,e0\,s20 - \\
&2\,e0^2\,s20 - 7\,e0\,kd1\,s20 - 2\,ace50\,s20^2 + \\
&5\,e0\,s20^2 - 2\,kd1\,s20^2 - 2\,s20^3) + \\
&216\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2 \\
&(ace50\,e0^2 + 4\,e0^2\,kd1 - ace50\,e0\,s20 - \\
&2\,e0^2\,s20 - 7\,e0\,kd1\,s20 - 2\,ace50\,s20^2 + \\
&5\,e0\,s20^2 - 2\,kd1\,s20^2 - 2\,s20^3)^2 + \\
&\sqrt{(-4\,((-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2} \\
&e0^3 + 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0 \\
&kd1^2 + 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 +
\end{aligned}
$$

FIG. 6P

$$5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + 4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2)^2 + 24\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2\,(2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3) + 12\,kd1\,(4\,ace50^2 - 5\,ace50\,e0 + 2\,e0^2 - 2\,ace50\,kd1 + 4\,e0\,kd1 - 2\,kd1^2 - 4\,ace50\,s20 + 2\,e0\,s20 + 6\,kd1\,s20)\,(ace50\,e0^2 + 4\,e0^2\,kd1 - ace50\,e0\,s20 - 2\,e0^2\,s20 - 7\,e0\,kd1\,s20 - 2\,ace50\,s20^2 + 5\,e0\,s20^2 - 2\,kd1\,s20^2 - 2\,s20^3))^3 + (2\,(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + 4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2)^3 + 108\,kd1^2\,(4\,ace50^2 - 5\,ace50\,e0 + 2\,e0^2 - 2\,ace50\,kd1 + 4\,e0\,kd1 - 2\,kd1^2 - 4\,ace50\,s20 + 2\,e0\,s20 + 6\,kd1\,s20)^2\,(2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3) - 144\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2\,(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + 5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + 4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2)\,(2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3) + 36\,kd1\,(4\,ace50^2 - 5\,ace50\,e0 + 2\,e0^2 - 2\,ace50$$

FIG. 6Q

$$
\begin{aligned}
&\text{kd1} + 4\,\text{e0}\,\text{kd1} - 2\,\text{kd1}^2 - 4\,\text{ace50}\,\text{s20} + \\
&2\,\text{e0}\,\text{s20} + 6\,\text{kd1}\,\text{s20})\,(-4\,\text{ace50}^2\,\text{e0} + \\
&6\,\text{ace50}\,\text{e0}^2 - 2\,\text{e0}^3 + 6\,\text{ace50}\,\text{e0}\,\text{kd1} - 4 \\
&\text{e0}^2\,\text{kd1} + 10\,\text{e0}\,\text{kd1}^2 + 8\,\text{ace50}^2\,\text{s20} - 14 \\
&\text{ace50}\,\text{e0}\,\text{s20} + 5\,\text{e0}^2\,\text{s20} + 2\,\text{e0}\,\text{kd1}\,\text{s20} + \\
&4\,\text{kd1}^2\,\text{s20} + 4\,\text{ace50}\,\text{s20}^2 - 2\,\text{e0}\,\text{s20}^2 - \\
&12\,\text{kd1}\,\text{s20}^2)\,(\text{ace50}\,\text{e0}^2 + 4\,\text{e0}^2\,\text{kd1} - \\
&\text{ace50}\,\text{e0}\,\text{s20} - 2\,\text{e0}^2\,\text{s20} - 7\,\text{e0}\,\text{kd1}\,\text{s20} - \\
&2\,\text{ace50}\,\text{s20}^2 + 5\,\text{e0}\,\text{s20}^2 - 2\,\text{kd1}\,\text{s20}^2 - \\
&2\,\text{s20}^3) + 216\,(2\,\text{ace50} - \text{e0} - 2\,\text{kd1})\,\text{kd1}^2 \\
&(\text{ace50}\,\text{e0}^2 + 4\,\text{e0}^2\,\text{kd1} - \text{ace50}\,\text{e0}\,\text{s20} - \\
&2\,\text{e0}^2\,\text{s20} - 7\,\text{e0}\,\text{kd1}\,\text{s20} - 2\,\text{ace50}\,\text{s20}^2 + \\
&5\,\text{e0}\,\text{s20}^2 - 2\,\text{kd1}\,\text{s20}^2 - 2\,\text{s20}^3)^2)^2))^{1/3}) + \\
&\frac{1}{6\,2^{1/3}\,(2\,\text{ace50} - \text{e0} - 2\,\text{kd1})}((2\,(-4\,\text{ace50}^2\,\text{e0} + \\
&6\,\text{ace50}\,\text{e0}^2 - 2\,\text{e0}^3 + 6\,\text{ace50}\,\text{e0}\,\text{kd1} - \\
&4\,\text{e0}^2\,\text{kd1} + 10\,\text{e0}\,\text{kd1}^2 + 8\,\text{ace50}^2\,\text{s20} - \\
&14\,\text{ace50}\,\text{e0}\,\text{s20} + 5\,\text{e0}^2\,\text{s20} + \\
&2\,\text{e0}\,\text{kd1}\,\text{s20} + 4\,\text{kd1}^2\,\text{s20} + 4\,\text{ace50} \\
&\text{s20}^2 - 2\,\text{e0}\,\text{s20}^2 - 12\,\text{kd1}\,\text{s20}^2)^3 + \\
&108\,\text{kd1}^2\,(4\,\text{ace50}^2 - 5\,\text{ace50}\,\text{e0} + \\
&2\,\text{e0}^2 - 2\,\text{ace50}\,\text{kd1} + 4\,\text{e0}\,\text{kd1} - 2\,\text{kd1}^2 - \\
&4\,\text{ace50}\,\text{s20} + 2\,\text{e0}\,\text{s20} + 6\,\text{kd1}\,\text{s20})^2 \\
&(2\,\text{e0}^3 - 9\,\text{e0}^2\,\text{s20} + 12\,\text{e0}\,\text{s20}^2 - 4\,\text{s20}^3) - \\
&144\,(2\,\text{ace50} - \text{e0} - 2\,\text{kd1})\,\text{kd1}^2 \\
&(-4\,\text{ace50}^2\,\text{e0} + 6\,\text{ace50}\,\text{e0}^2 - 2\,\text{e0}^3 + \\
&6\,\text{ace50}\,\text{e0}\,\text{kd1} - 4\,\text{e0}^2\,\text{kd1} + 10\,\text{e0}\,\text{kd1}^2 + \\
&8\,\text{ace50}^2\,\text{s20} - 14\,\text{ace50}\,\text{e0}\,\text{s20} +
\end{aligned}
$$

FIG. 6R

$$
\begin{aligned}
&5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + \\
&4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\big) \\
&\big(2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3\big) + \\
&36\,kd1\,\big(4\,ace50^2 - 5\,ace50\,e0 + 2\,e0^2 - \\
&2\,ace50\,kd1 + 4\,e0\,kd1 - 2\,kd1^2 - \\
&4\,ace50\,s20 + 2\,e0\,s20 + 6\,kd1\,s20\big) \\
&\big(-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2\,e0^3 + \\
&6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0\,kd1^2 + \\
&8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
&5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + \\
&4\,ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\big) \\
&\big(ace50\,e0^2 + 4\,e0^2\,kd1 - ace50\,e0\,s20 - \\
&2\,e0^2\,s20 - 7\,e0\,kd1\,s20 - 2\,ace50\,s20^2 + \\
&5\,e0\,s20^2 - 2\,kd1\,s20^2 - 2\,s20^3\big) + \\
&216\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2 \\
&\big(ace50\,e0^2 + 4\,e0^2\,kd1 - ace50\,e0\,s20 - \\
&2\,e0^2\,s20 - 7\,e0\,kd1\,s20 - 2\,ace50\,s20^2 + \\
&5\,e0\,s20^2 - 2\,kd1\,s20^2 - 2\,s20^3\big)^2 + \\
&\sqrt{\Big(-4\,\big((-4\,ace50^2\,e0 + 6\,ace50\,e0^2 - 2 \\
&e0^3 + 6\,ace50\,e0\,kd1 - 4\,e0^2\,kd1 + 10\,e0 \\
&kd1^2 + 8\,ace50^2\,s20 - 14\,ace50\,e0\,s20 + \\
&5\,e0^2\,s20 + 2\,e0\,kd1\,s20 + 4\,kd1^2\,s20 + 4 \\
&ace50\,s20^2 - 2\,e0\,s20^2 - 12\,kd1\,s20^2\big)^2 + \\
&24\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2 \\
&\big(2\,e0^3 - 9\,e0^2\,s20 + 12\,e0\,s20^2 - 4\,s20^3\big) + \\
&12\,kd1\,\big(4\,ace50^2 - 5\,ace50\,e0 + \\
&2\,e0^2 - 2\,ace50\,kd1 + 4\,e0\,kd1 - 2\,kd1^2 - \\
&4\,ace50\,s20 + 2\,e0\,s20 + 6\,kd1\,s20\big)
\end{aligned}
$$

FIG. 6S

$$
\begin{aligned}
&(\mathrm{ace50\,e0^2} + 4\,\mathrm{e0^2\,kd1} - \mathrm{ace50\,e0\,s20} - \\
&2\,\mathrm{e0^2\,s20} - 7\,\mathrm{e0\,kd1\,s20} - 2\,\mathrm{ace50\,s20^2} + \\
&5\,\mathrm{e0\,s20^2} - 2\,\mathrm{kd1\,s20^2} - 2\,\mathrm{s20^3}))^3 + \\
&(2(-4\,\mathrm{ace50^2\,e0} + 6\,\mathrm{ace50\,e0^2} - 2 \\
&\mathrm{e0^3} + 6\,\mathrm{ace50\,e0\,kd1} - 4\,\mathrm{e0^2\,kd1} + 10\,\mathrm{e0} \\
&\mathrm{kd1^2} + 8\,\mathrm{ace50^2\,s20} - 14\,\mathrm{ace50\,e0\,s20} + \\
&5\,\mathrm{e0^2\,s20} + 2\,\mathrm{e0\,kd1\,s20} + 4\,\mathrm{kd1^2\,s20} + 4 \\
&\mathrm{ace50\,s20^2} - 2\,\mathrm{e0\,s20^2} - 12\,\mathrm{kd1\,s20^2})^3 + \\
&108\,\mathrm{kd1^2}(4\,\mathrm{ace50^2} - 5\,\mathrm{ace50\,e0} + \\
&2\,\mathrm{e0^2} - 2\,\mathrm{ace50\,kd1} + 4\,\mathrm{e0\,kd1} - 2\,\mathrm{kd1^2} - \\
&4\,\mathrm{ace50\,s20} + 2\,\mathrm{e0\,s20} + 6\,\mathrm{kd1\,s20})^2 \\
&(2\,\mathrm{e0^3} - 9\,\mathrm{e0^2\,s20} + 12\,\mathrm{e0\,s20^2} - \\
&4\,\mathrm{s20^3}) - 144\,(2\,\mathrm{ace50} - \mathrm{e0} - 2\,\mathrm{kd1})\,\mathrm{kd1^2} \\
&(-4\,\mathrm{ace50^2\,e0} + 6\,\mathrm{ace50\,e0^2} - 2\,\mathrm{e0^3} + \\
&6\,\mathrm{ace50\,e0\,kd1} - 4\,\mathrm{e0^2\,kd1} + 10\,\mathrm{e0\,kd1^2} + \\
&8\,\mathrm{ace50^2\,s20} - 14\,\mathrm{ace50\,e0\,s20} + \\
&5\,\mathrm{e0^2\,s20} + 2\,\mathrm{e0\,kd1\,s20} + 4\,\mathrm{kd1^2\,s20} + \\
&4\,\mathrm{ace50\,s20^2} - 2\,\mathrm{e0\,s20^2} - 12\,\mathrm{kd1\,s20^2}) \\
&(2\,\mathrm{e0^3} - 9\,\mathrm{e0^2\,s20} + 12\,\mathrm{e0\,s20^2} - 4\,\mathrm{s20^3}) + \\
&36\,\mathrm{kd1}(4\,\mathrm{ace50^2} - 5\,\mathrm{ace50\,e0} + \\
&2\,\mathrm{e0^2} - 2\,\mathrm{ace50\,kd1} + 4\,\mathrm{e0\,kd1} - 2\,\mathrm{kd1^2} - \\
&4\,\mathrm{ace50\,s20} + 2\,\mathrm{e0\,s20} + 6\,\mathrm{kd1\,s20}) \\
&(-4\,\mathrm{ace50^2\,e0} + 6\,\mathrm{ace50\,e0^2} - 2\,\mathrm{e0^3} + \\
&6\,\mathrm{ace50\,e0\,kd1} - 4\,\mathrm{e0^2\,kd1} + 10\,\mathrm{e0\,kd1^2} + \\
&8\,\mathrm{ace50^2\,s20} - 14\,\mathrm{ace50\,e0\,s20} + \\
&5\,\mathrm{e0^2\,s20} + 2\,\mathrm{e0\,kd1\,s20} + 4\,\mathrm{kd1^2\,s20} + \\
&4\,\mathrm{ace50\,s20^2} - 2\,\mathrm{e0\,s20^2} - 12\,\mathrm{kd1\,s20^2}) \\
&(\mathrm{ace50\,e0^2} + 4\,\mathrm{e0^2\,kd1} - \mathrm{ace50\,e0\,s20} -
\end{aligned}
$$

FIG. 6T

$$2\,e0^2\,s20 - 7\,e0\,kd1\,s20 - 2\,ace50\,s20^2 + 5\,e0\,s20^2 - 2\,kd1\,s20^2 - 2\,s20^3) + 216\,(2\,ace50 - e0 - 2\,kd1)\,kd1^2\,(ace50\,e0^2 + 4\,e0^2\,kd1 - ace50\,e0\,s20 - 2\,e0^2\,s20 - 7\,e0\,kd1\,s20 - 2\,ace50\,s20^2 + 5\,e0\,s20^2 - 2\,kd1\,s20^2 - 2\,s20^3)^2)^2))^{1/3})))$$

FIG. 6U

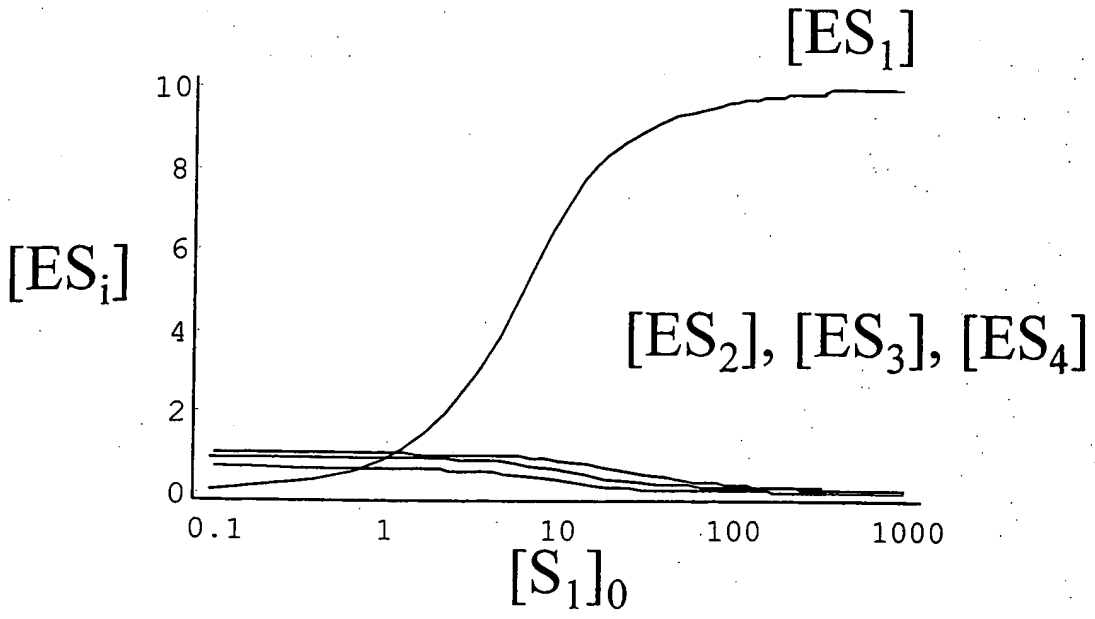



FIG. 7

$K_{d1} = 1\,\mu M$

$K_{d2} = 0.5$

$K_{d3} = 2$

$K_{d4} = 5$

$[S_1]$ = variable

$[S_2]_0 = [S_3]_0 = [S_4]_0 = 1$

$[E]_0 = 10$

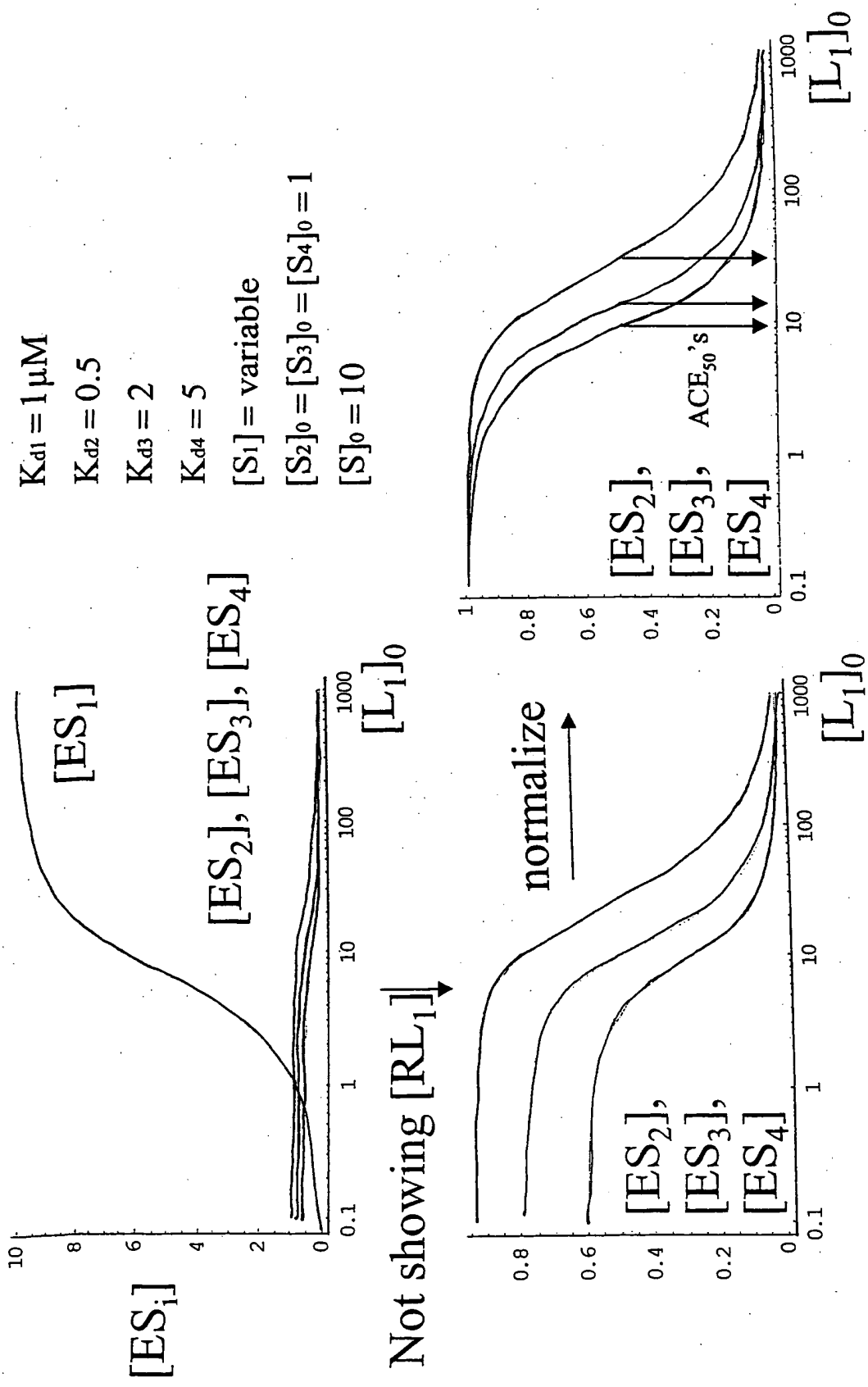

$K_{d1} = 1\,\mu M$
$K_{d2} = 0.5$
$K_{d3} = 2$
$K_{d4} = 5$
$[S_1]$ = variable
$[S_2]_0 = [S_3]_0 = [S_4]_0 = 1$
$[S]_0 = 10$
$[ES_i]$
$[ES_1]$
$[ES_2]$, $[ES_3]$, $[ES_4]$
$[L_1]_0$
Not showing $[RL_1]$
normalize
$[ES_2]$, $[ES_3]$, $[ES_4]$
$ACE_{50}$'s

FIG. 8

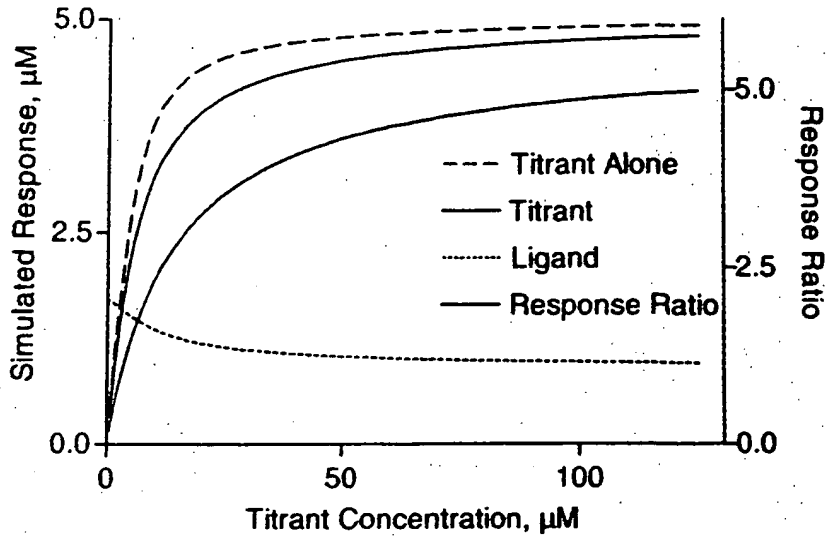

5.0
2.5
0.0
Simulated Response, µM
0
50
100
Titrant Concentration, µM
Titrant Alone
Titrant
Ligand
Response Ratio
5.0
2.5
0.0
Response Ratio

FIG. 9

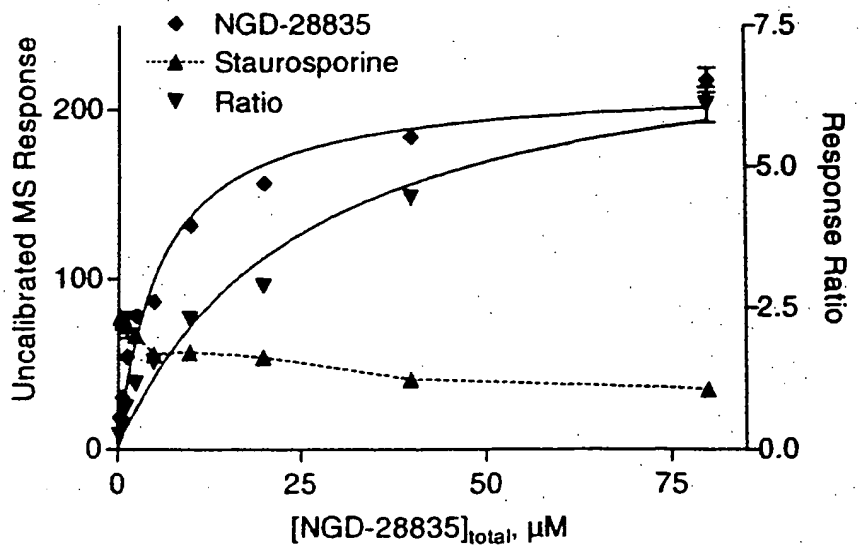

NGD-28835
Staurosporine
Ratio
200
100
0
Uncalibrated MS Response
0
25
50
75
[NGD-28835]total, µM
7.5
5.0
2.5
0.0
Response Ratio

FIG. 10

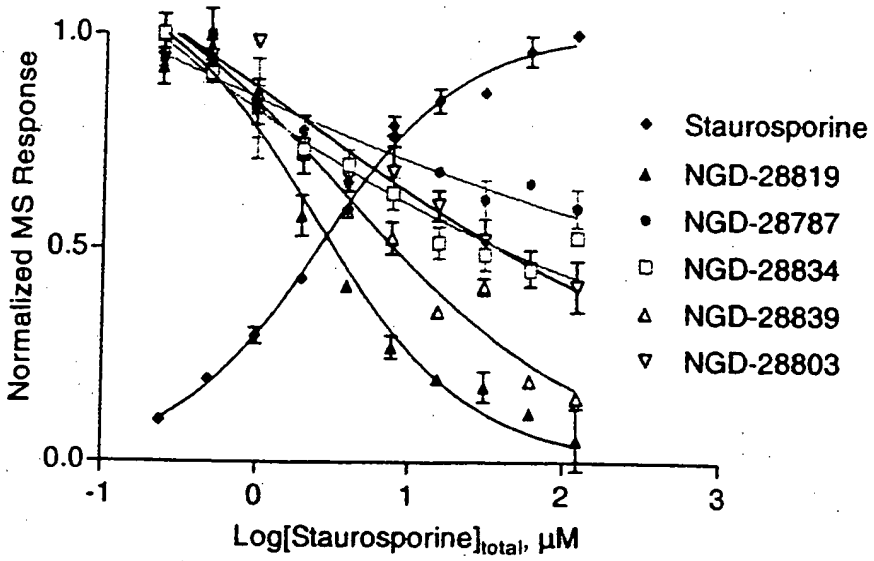



FIG. 11A

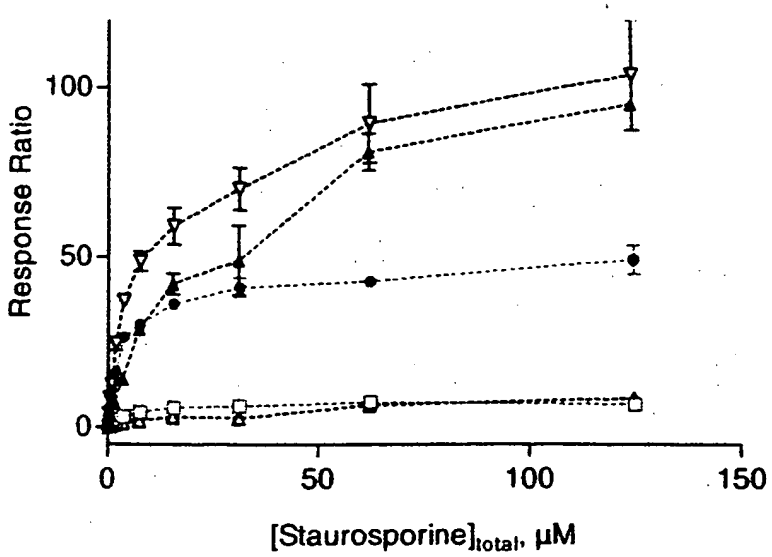



FIG. 11B

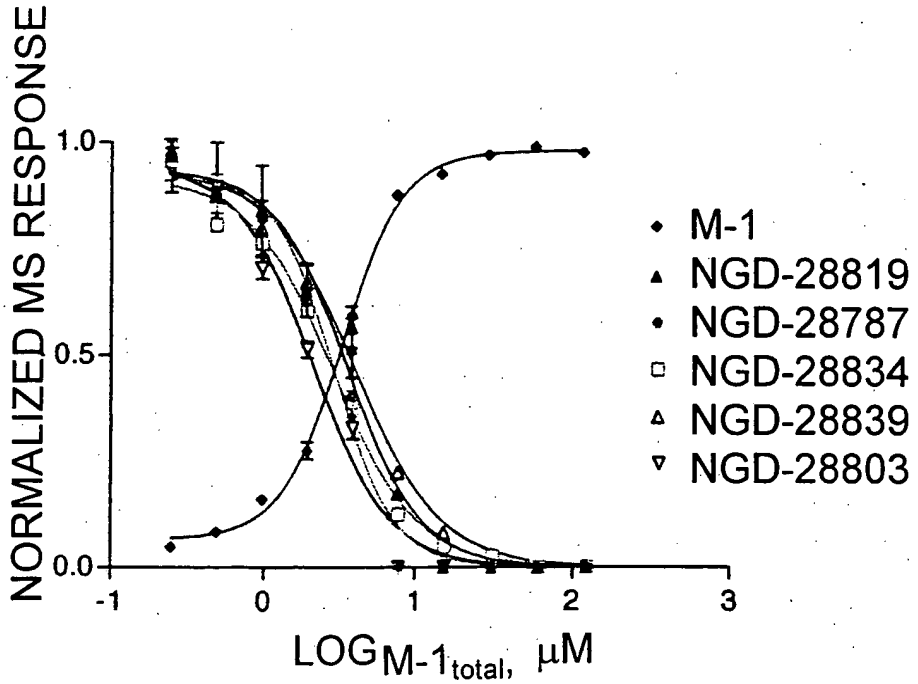



FIG. 11C

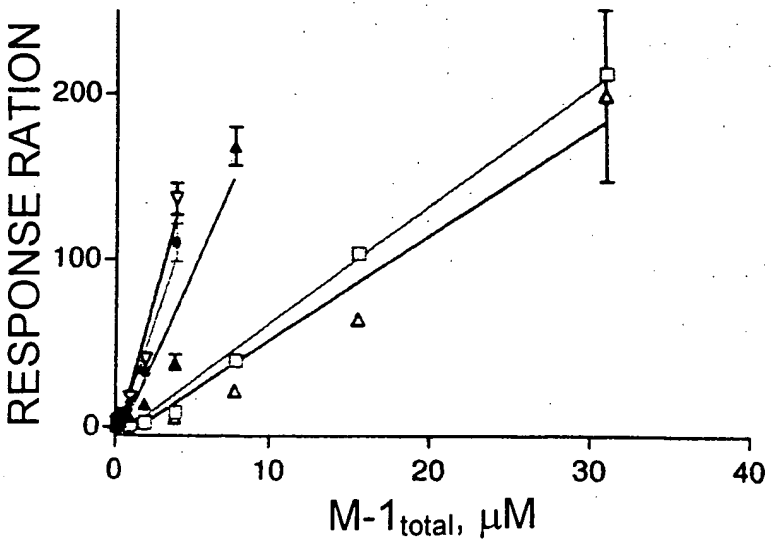



FIG. 11D

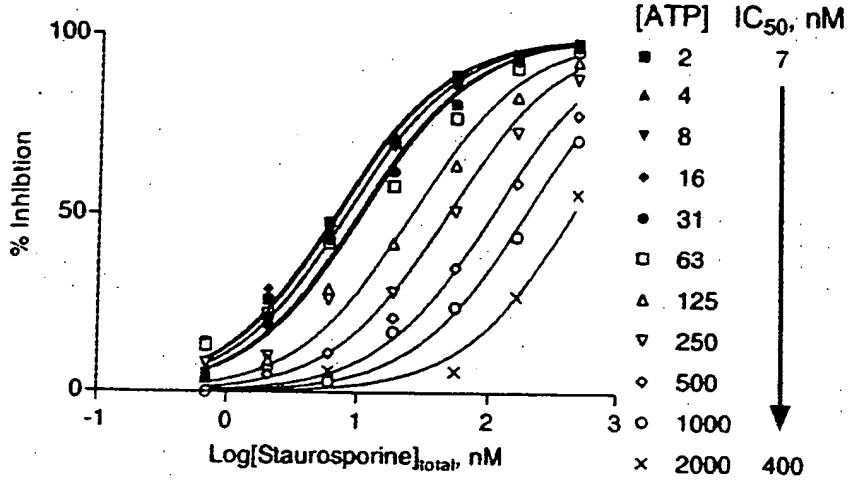



FIG. 12A

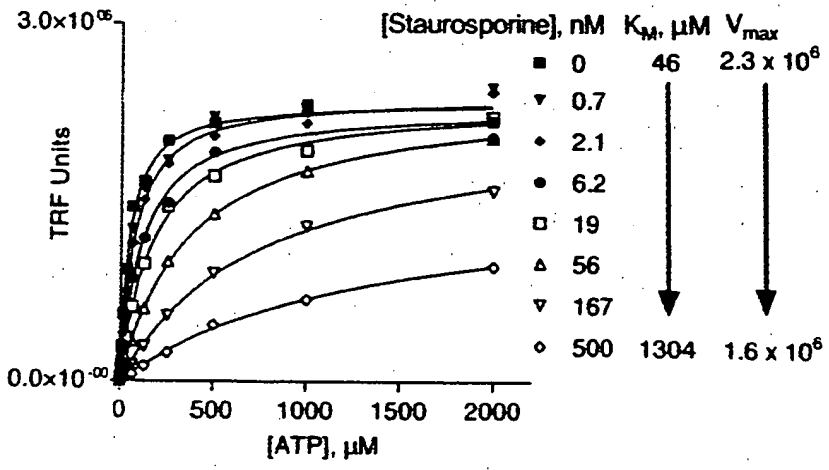



FIG. 12B

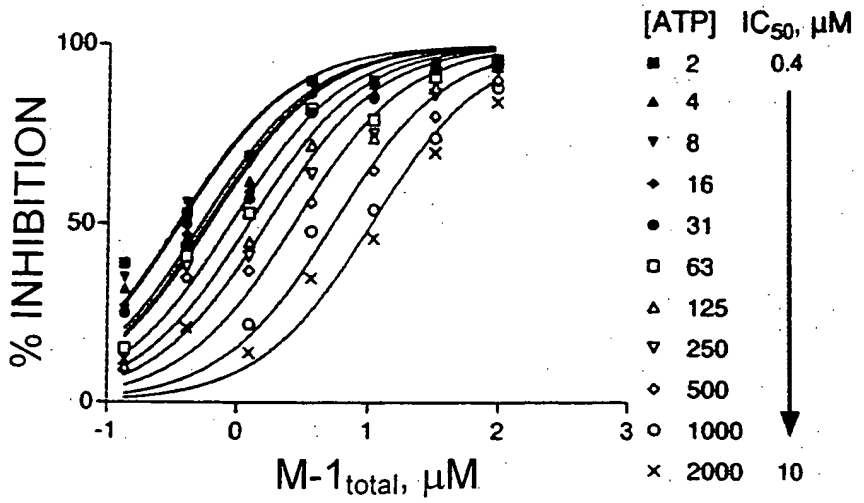



FIG. 12C

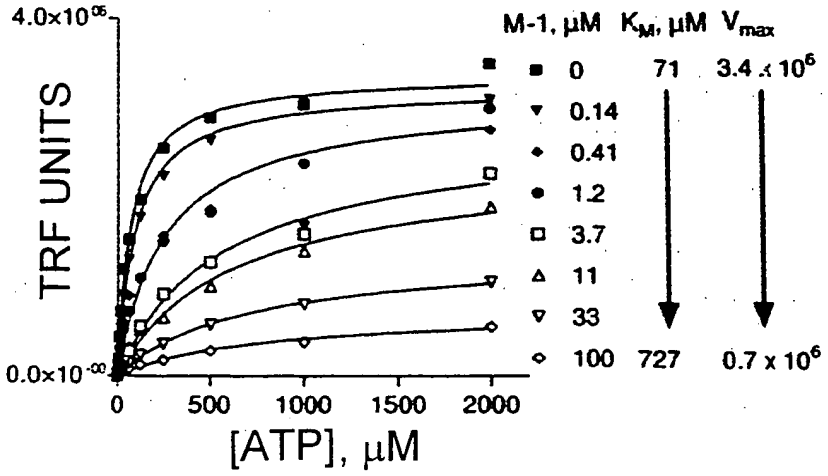



FIG. 12D

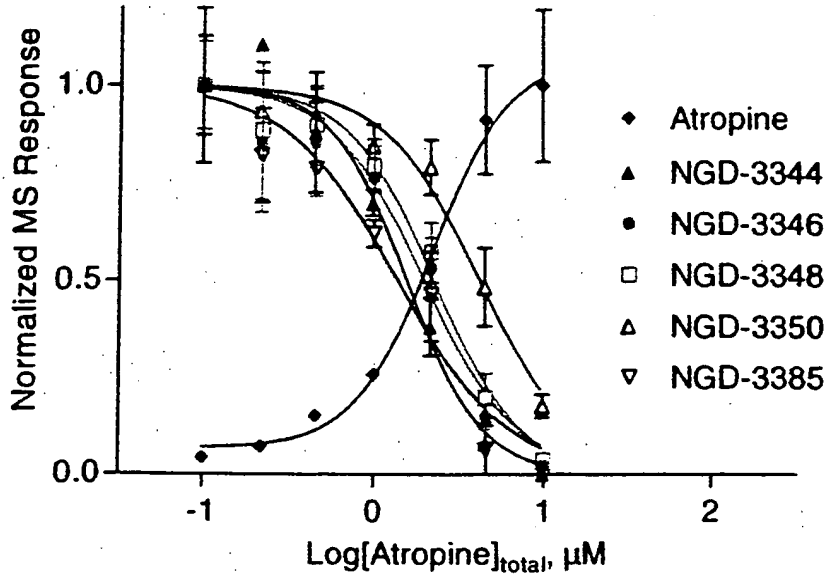



FIG. 13A

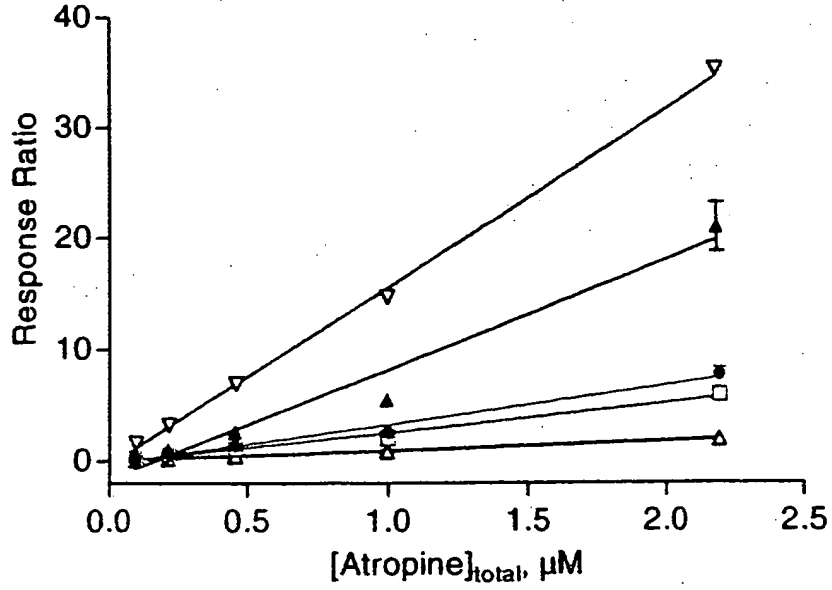



FIG. 13B

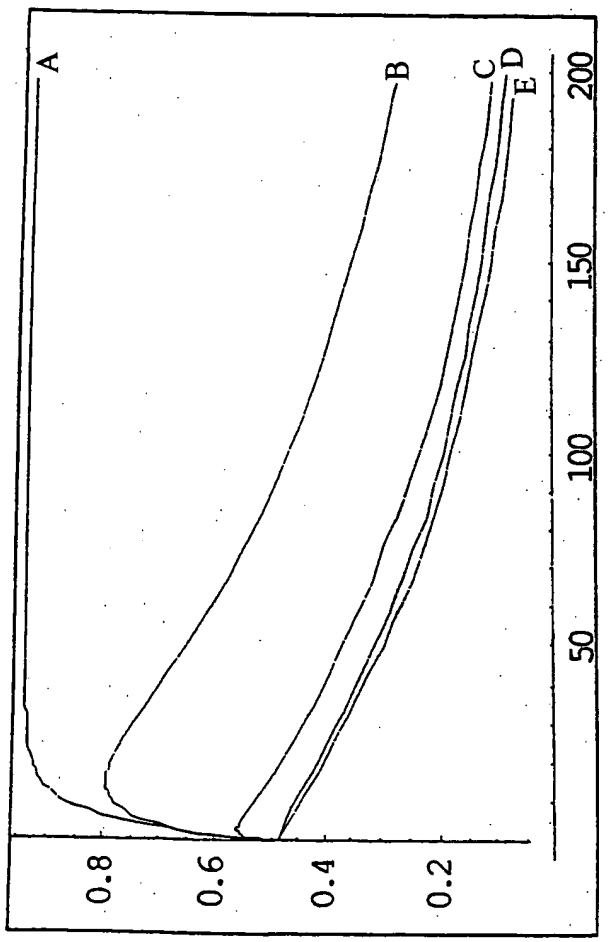

$E_0$= 5 µM (before dilution)
$s_0$=1 µM
$ks_1$=0.1 µM$^{-1}$sec$^{-1}$
$ks_2$=0.01 sec$^{-1}$
$I_0$ = 100 µM
$K_d(i)$= $K_d(s)$= 0.1 µM
$ki_1$= 0 µM$^{-1}$sec$^{-1}$ (A)
$ki_1$= 0.001 µM$^{-1}$sec$^{-1}$ (B)
$ki_1$= 0.01 µM$^{-1}$sec$^{-1}$ (C)
$ki_1$= 0.1 µM$^{-1}$sec$^{-1}$ (D)
Theoretical curve (E)
[E·S]
0.8
0.6
0.4
0.2
50
100
150
200
Time, sec
A
B
C
D
E

FIG. 14

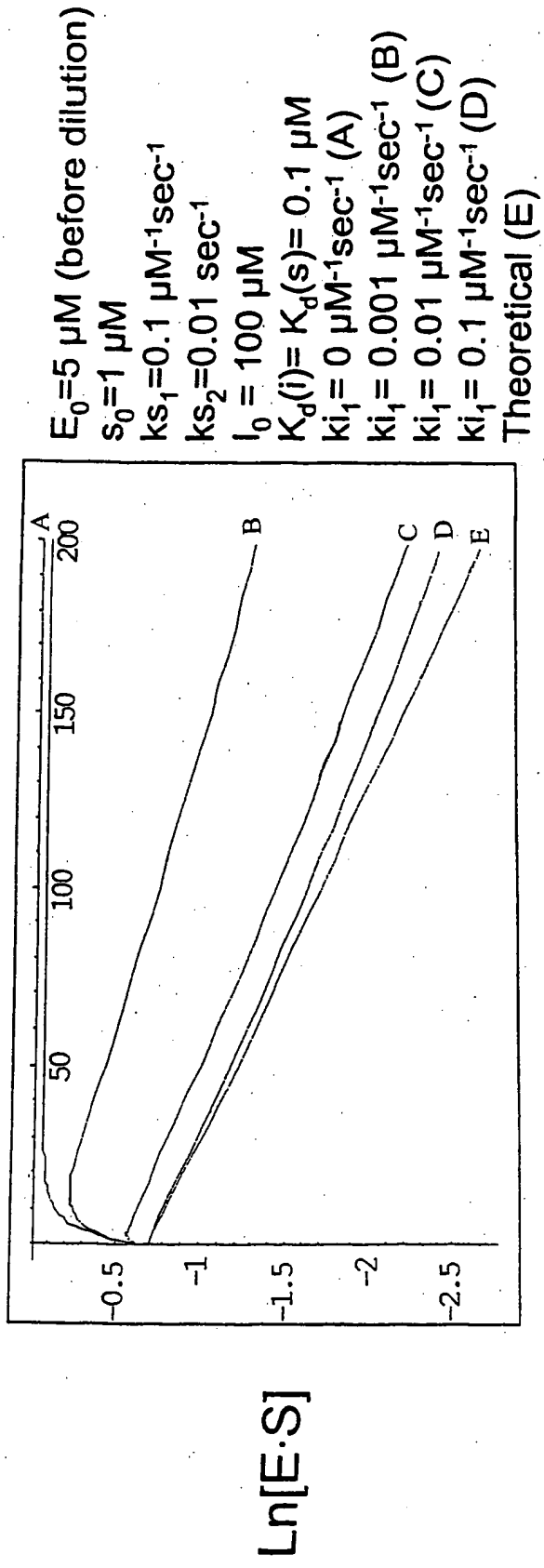

$E_0$=5 µM (before dilution)
$s_0$=1 µM
$ks_1$=0.1 µM$^{-1}$sec$^{-1}$
$ks_2$=0.01 sec$^{-1}$
$I_0$ = 100 µM
$K_d(i)$= $K_d(s)$= 0.1 µM
$ki_1$= 0 µM$^{-1}$sec$^{-1}$ (A)
$ki_1$= 0.001 µM$^{-1}$sec$^{-1}$ (B)
$ki_1$= 0.01 µM$^{-1}$sec$^{-1}$ (C)
$ki_1$= 0.1 µM$^{-1}$sec$^{-1}$ (D)
Theoretical (E)
A
B
C
D
E
50
100
150
200
-0.5
-1
-1.5
-2
-2.5
Ln[E·S]
Time, sec

FIG. 15

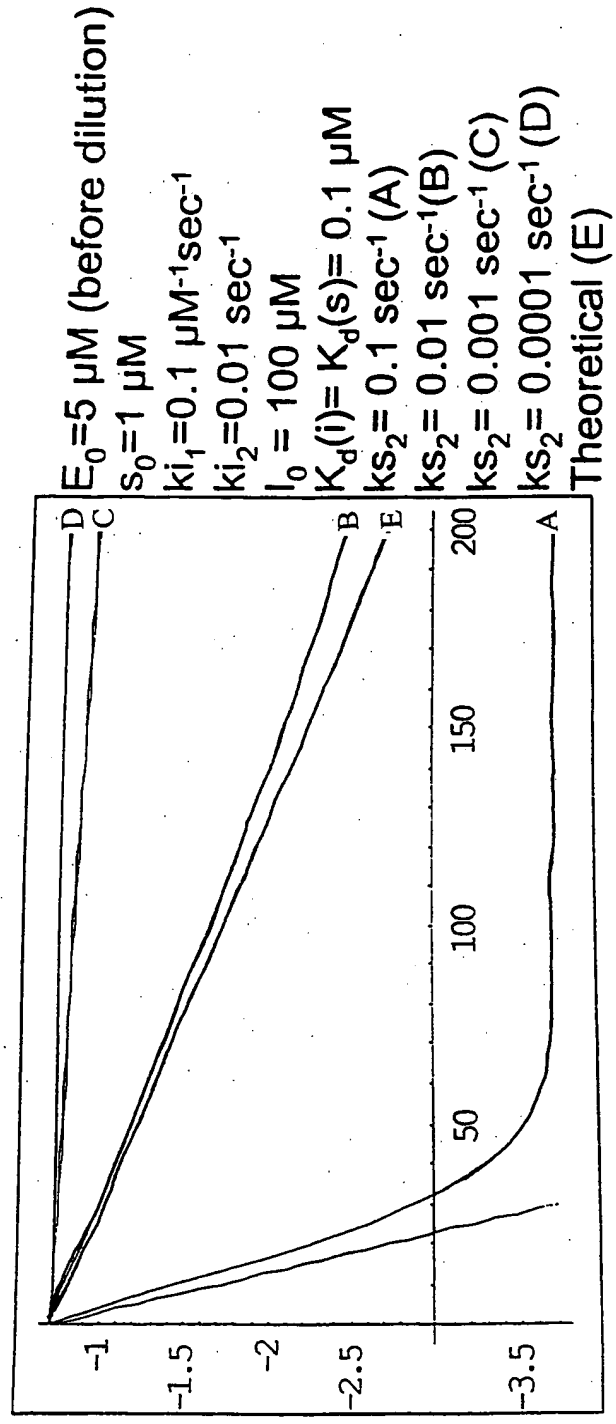

$E_0$=5 µM (before dilution)
$s_0$=1 µM
$ki_1$=0.1 µM$^{-1}$sec$^{-1}$
$ki_2$=0.01 sec$^{-1}$
$I_0$ = 100 µM
$K_d(i) = K_d(s)$= 0.1 µM
$ks_2$= 0.1 sec$^{-1}$ (A)
$ks_2$= 0.01 sec$^{-1}$(B)
$ks_2$= 0.001 sec$^{-1}$ (C)
$ks_2$= 0.0001 sec$^{-1}$ (D)
Theoretical (E)
D
C
B
E
A
-1
-1.5
-2
-2.5
-3.5
50
100
150
200
Ln[E·S]
Time, sec

FIG. 16

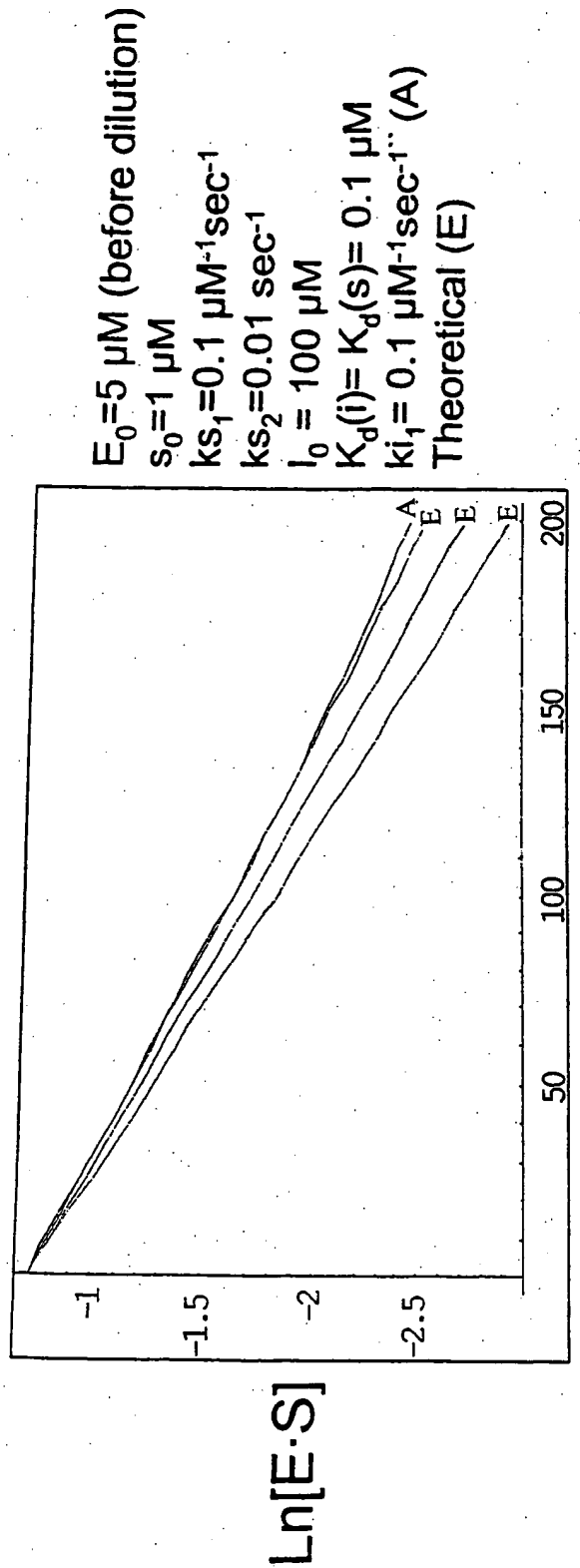

$E_0$=5 µM (before dilution)
$s_0$=1 µM
$ks_1$=0.1 µM$^{-1}$sec$^{-1}$
$ks_2$=0.01 sec$^{-1}$
$I_0$ = 100 µM
$K_d(i) = K_d(s)$= 0.1 µM
$ki_1$= 0.1 µM$^{-1}$sec$^{-1}$ (A)
Theoretical (E)
A
E
E
E
-1
-1.5
-2
-2.5
50
100
150
200
Ln[E·S]
Time, sec

FIG. 17

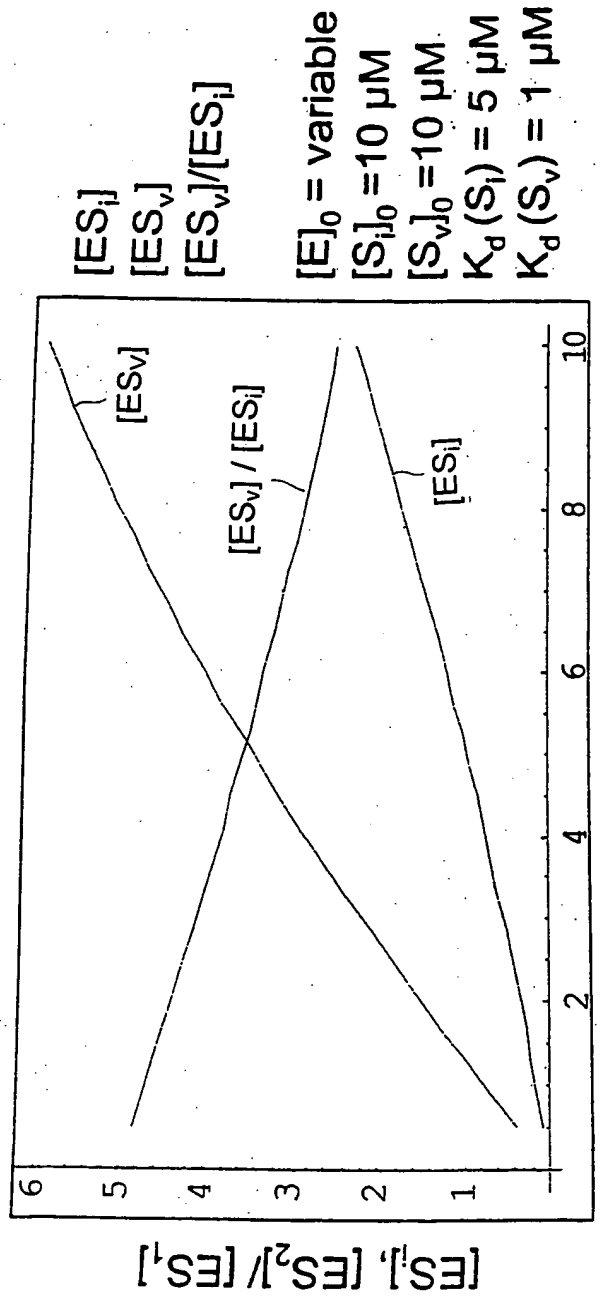

[ESi]
[ESv]
[ESv]/[ESi]
[E]0 = variable
[Si]0 =10 µM
[Sv]0 =10 µM
Kd (Si) = 5 µM
Kd (Sv) = 1 µM
[ESv]
[ESv] / [ESi]
[ESi]
[ESi], [ES2]/ [ES1]
[E]0, µM
1
2
3
4
5
6
2
4
6
8
10

FIG. 18

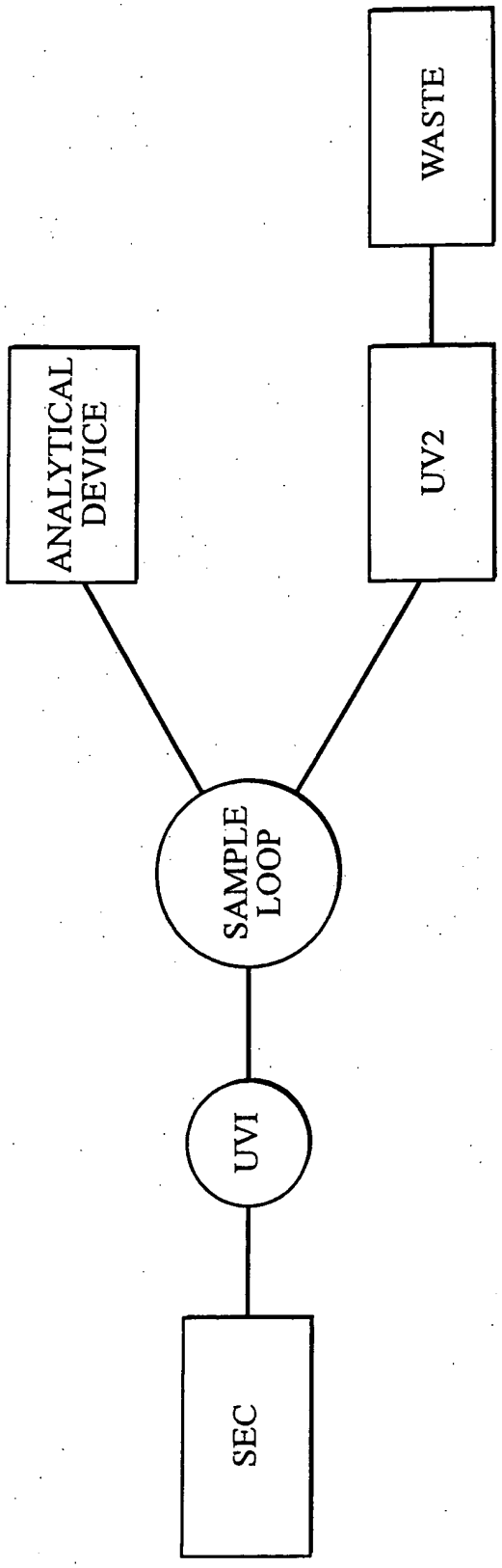



FIG. 19

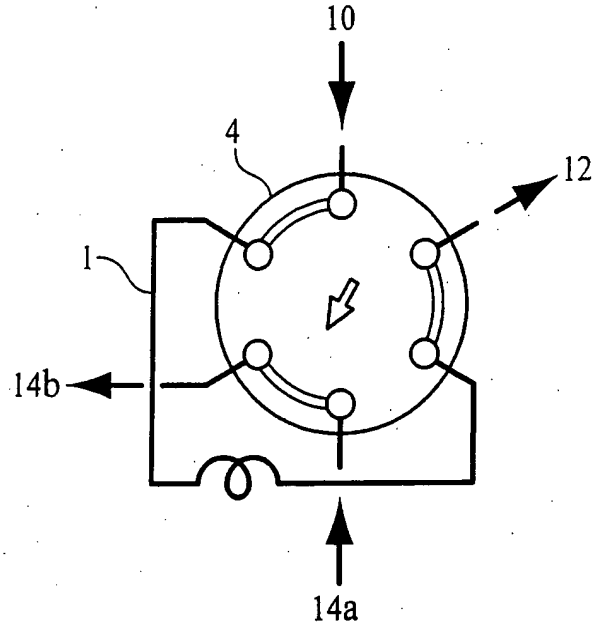



FIG. 20A

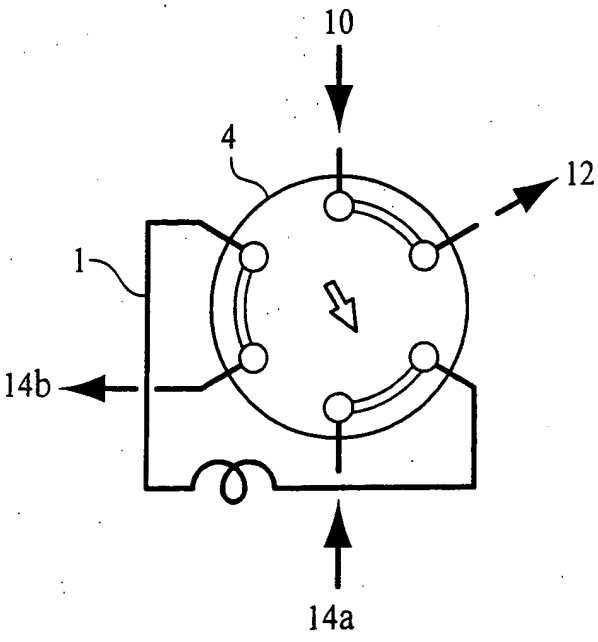



FIG. 20B

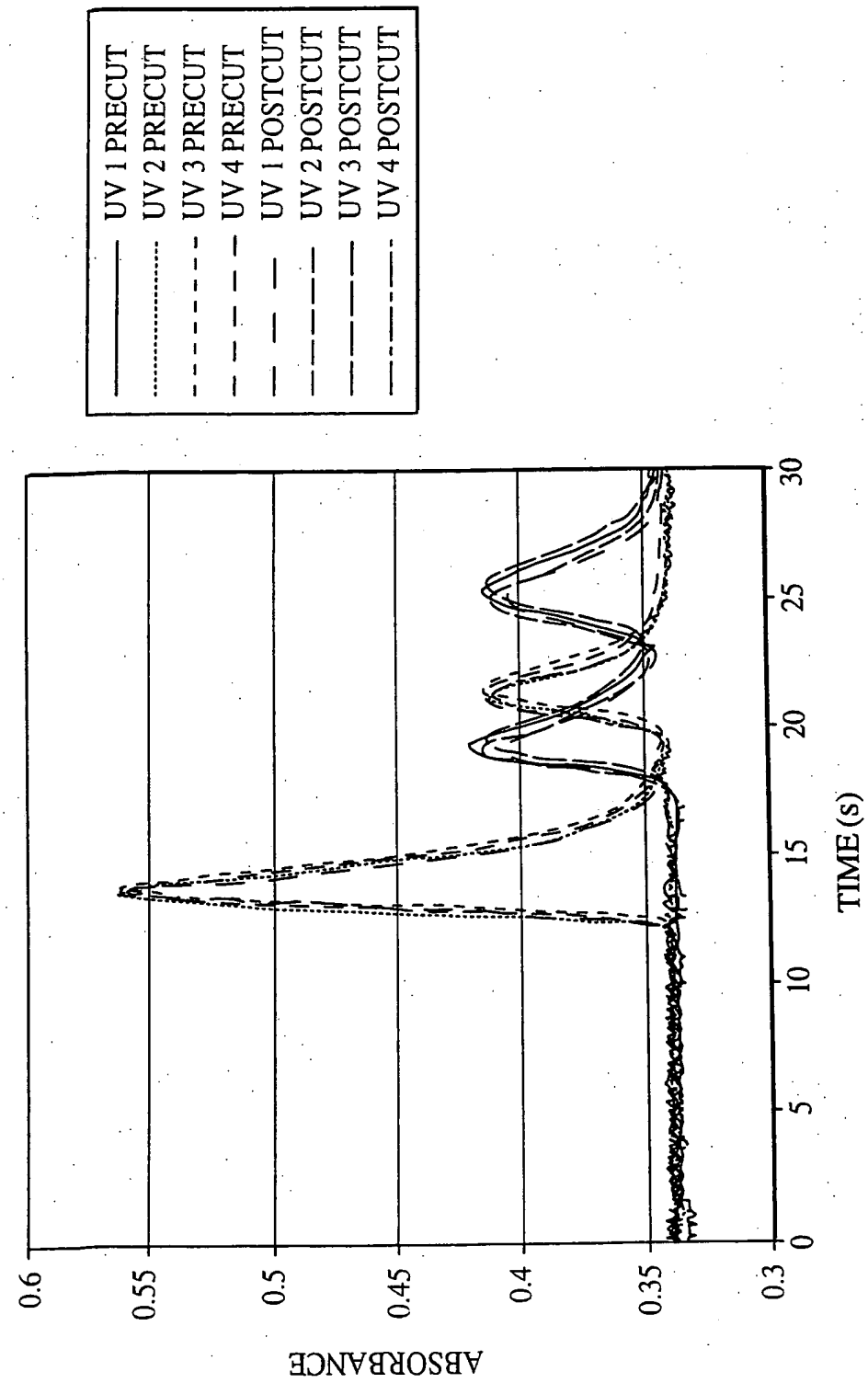

UV 1 PRECUT
UV 2 PRECUT
UV 3 PRECUT
UV 4 PRECUT
UV 1 POSTCUT
UV 2 POSTCUT
UV 3 POSTCUT
UV 4 POSTCUT
ABSORBANCE
0.6
0.55
0.5
0.45
0.4
0.35
0.3
0
5
10
15
20
25
30
TIME (s)

FIG. 21

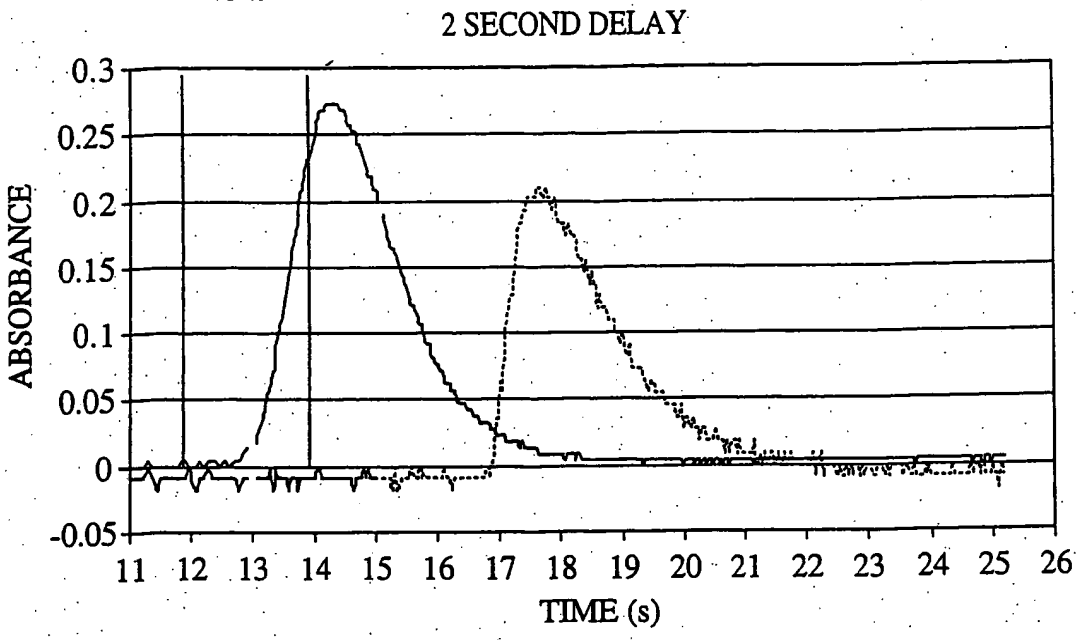



FIG. 22A

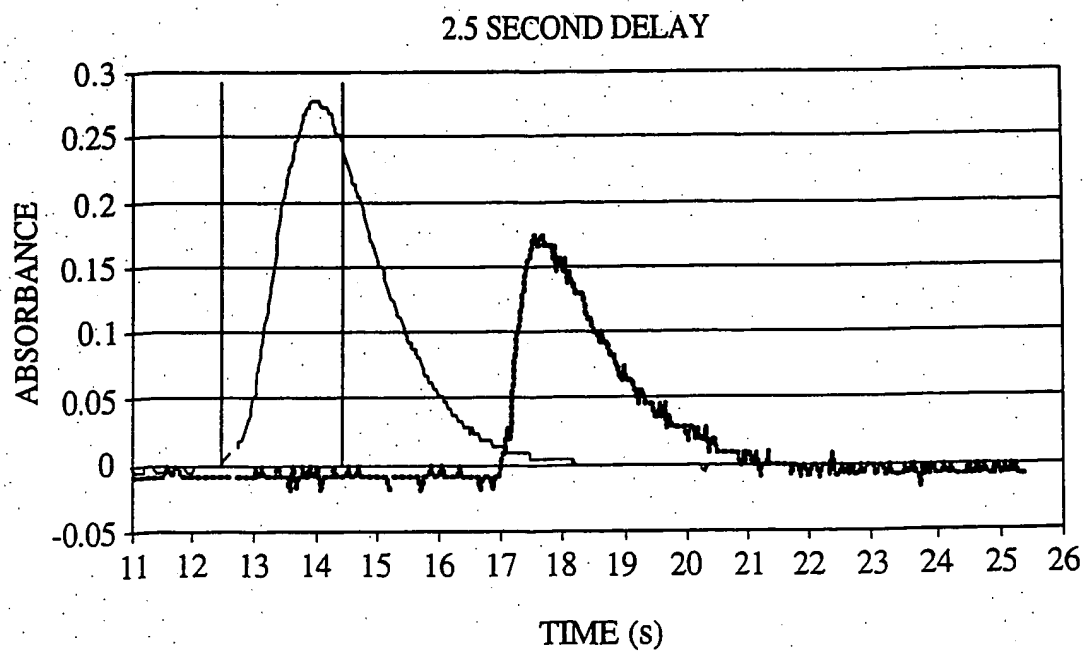



FIG. 22B

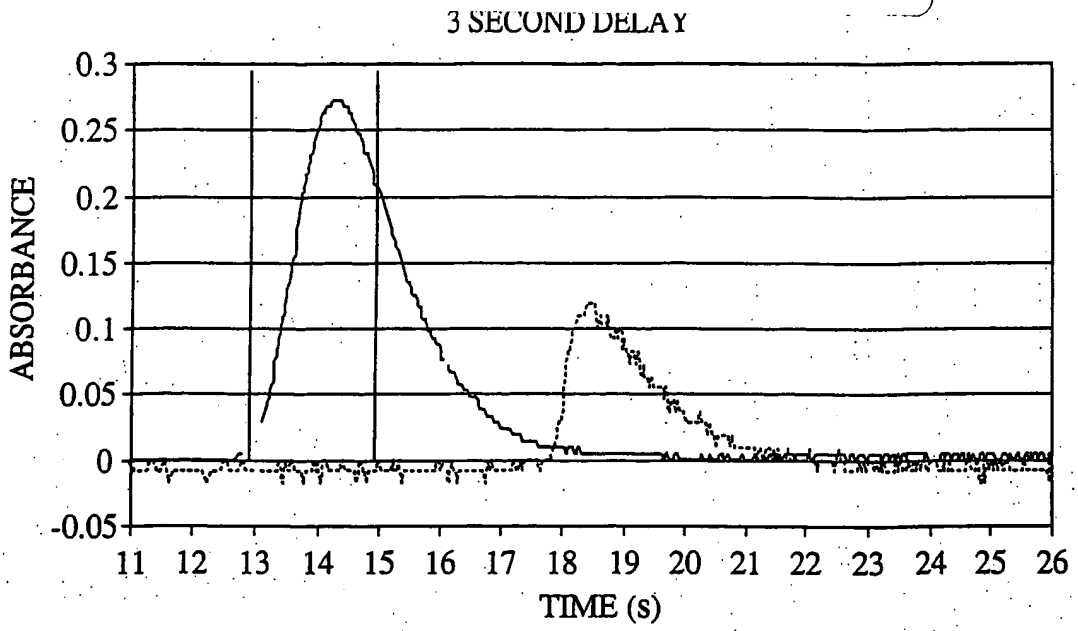



FIG. 22C

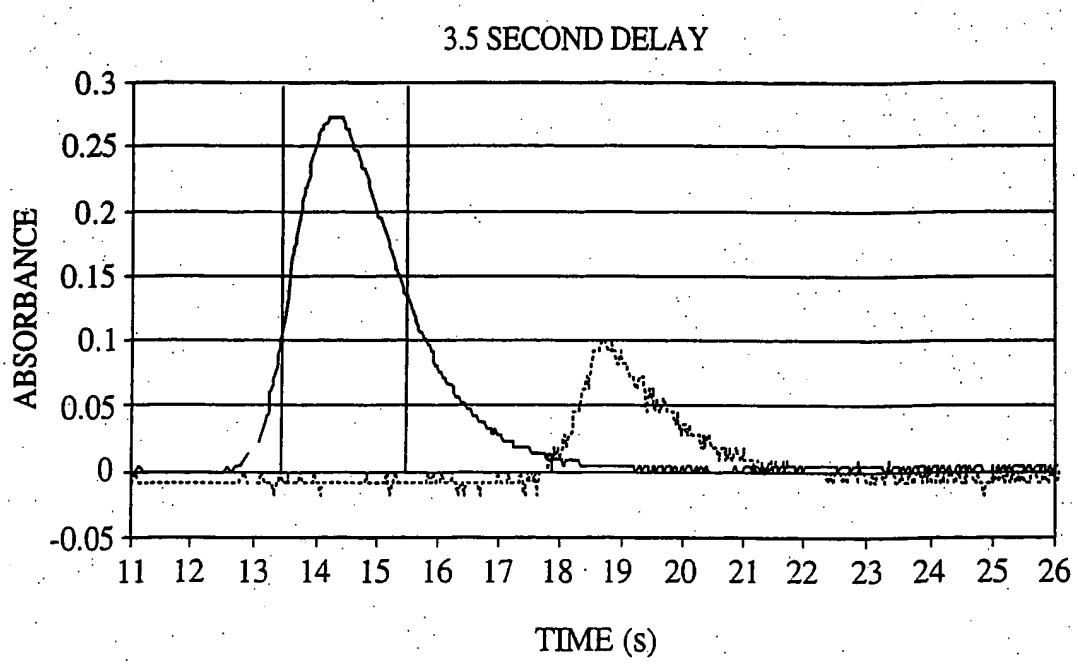



FIG. 22D

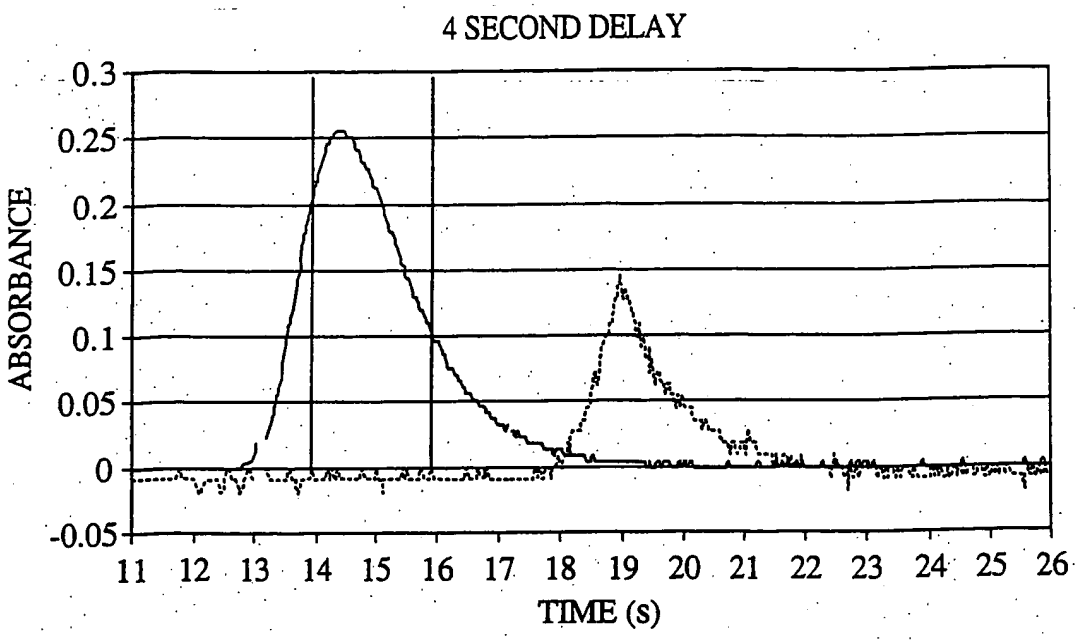



FIG. 22E

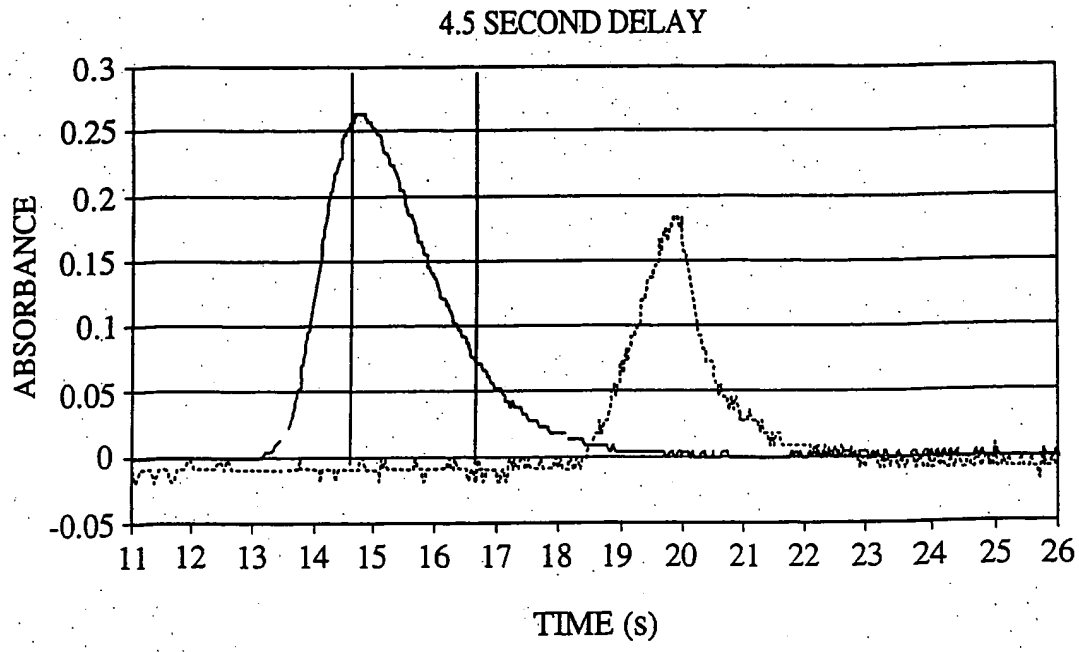



FIG. 22F

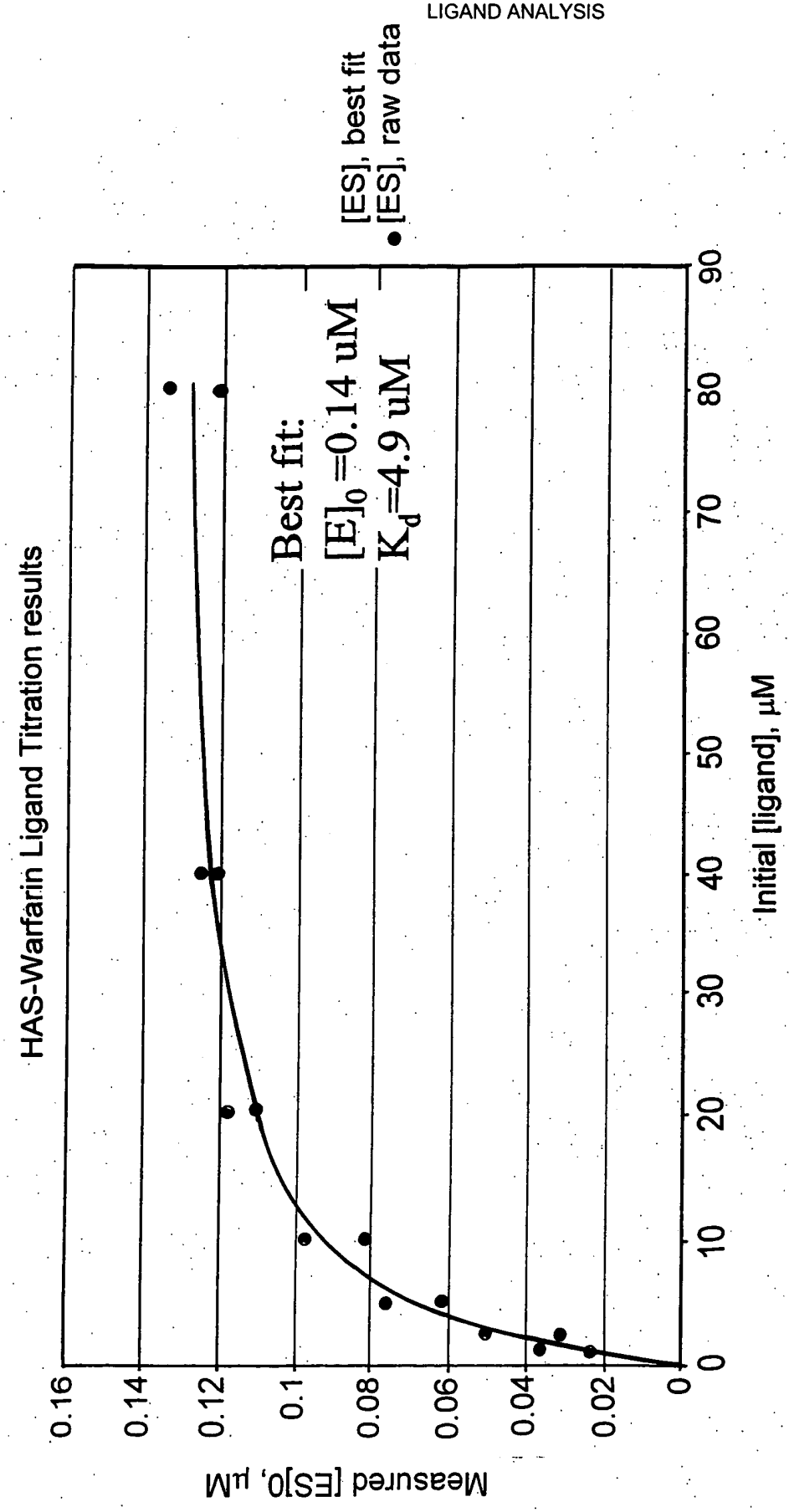

HAS-Warfarin Ligand Titration results
[ES], best fit
[ES], raw data
Best fit:
$[E]_0$ =0.14 uM
$K_d$=4.9 uM
Measured [ES]0, µM
Initial [ligand], µM
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
10
20
30
40
50
60
70
80
90

FIG. 23

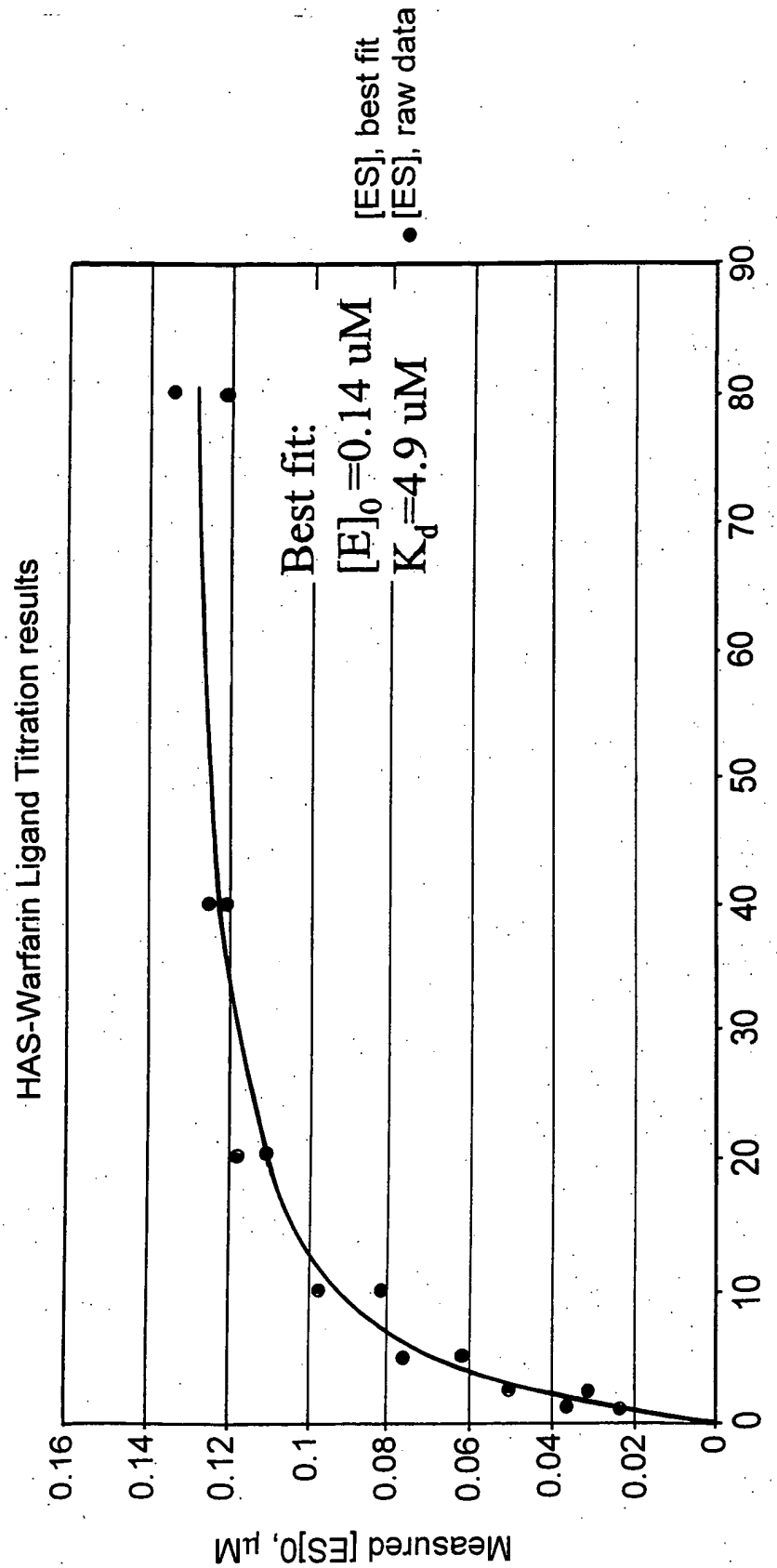

HAS-Warfarin Ligand Titration results
Best fit:
$[E]_0$ =0.14 uM
$K_d$=4.9 uM
[ES], best fit
[ES], raw data
Measured [ES]0, µM
Initial [ligand], µM
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0
10
20
30
40
50
60
70
80
90

FIG. 23

| | ACE50, µM |
|---|---|
| Atropine | 2.2 |
| NGD-3344 | 1.8 |
| NGD-3346 | 2.6 |
| NGD-3348 | 2.7 |
| NGD-3350 | 7.1 |
| NGD-3385 | 2.3 |

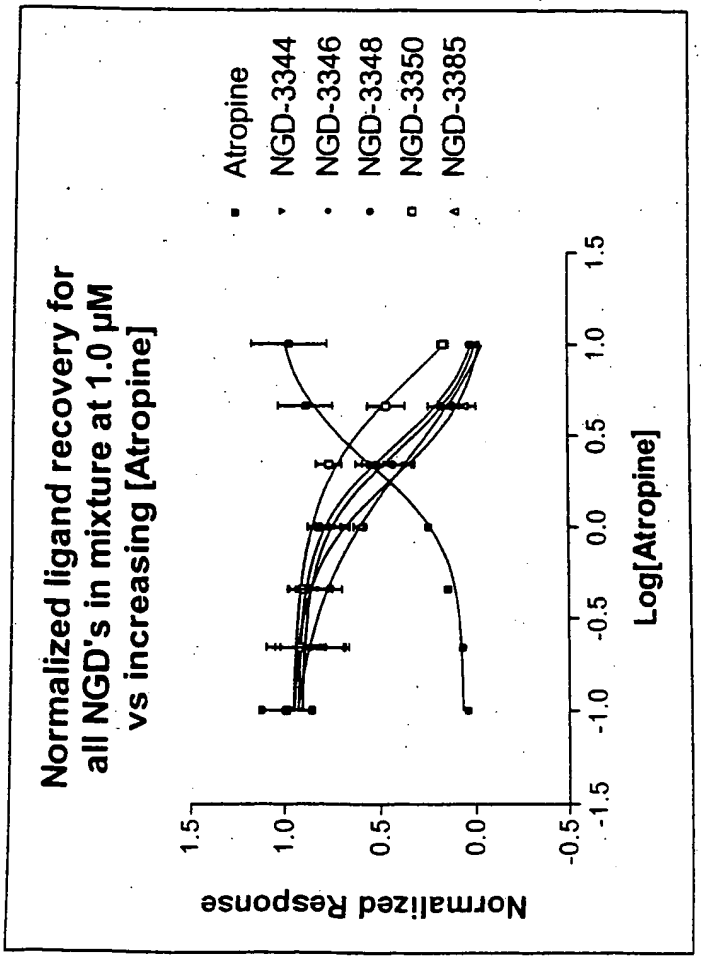
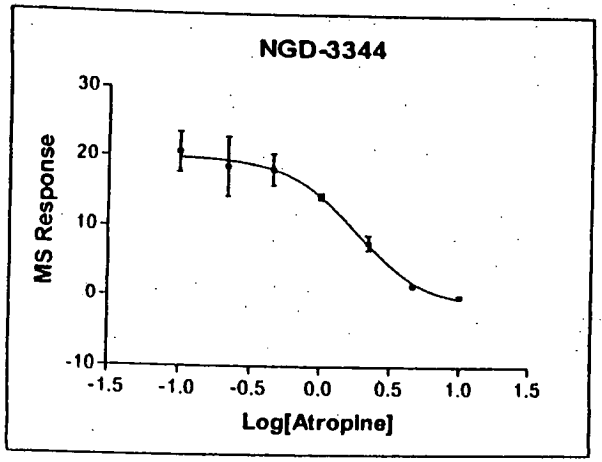
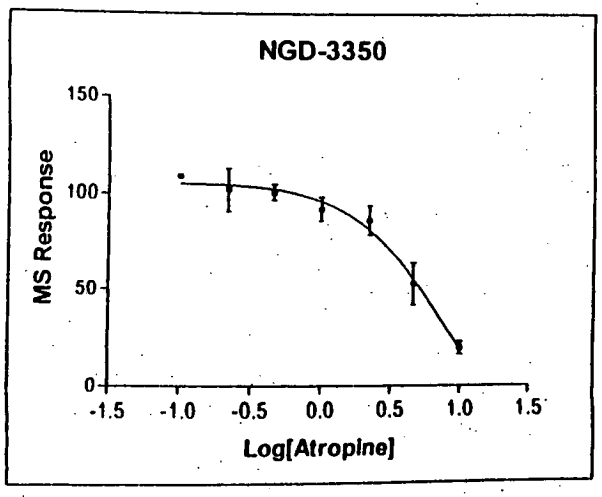



FIG. 24




- Comparison of NGD-3344 (weak) and NGD-3350 (strong) ligands shown
- $K_d$ of ligands in mixture calculated from $ACE_{50}$ given $K_d$ of inhibitor (0.010 µM) & protein concentration = 2.0 µM

| | ACE50, µM | Kd, µM |
|---|---|---|
| NGD-3344 | 1.8 | 0.75 |
| NGD-3346 | 2.6 | 0.20 |
| NGD-3348 | 2.7 | 0.19 |
| NGD-3350 | 7.1 | 0.03 |
| NGD-3385 | 2.3 | 0.30 |

FIG. 25

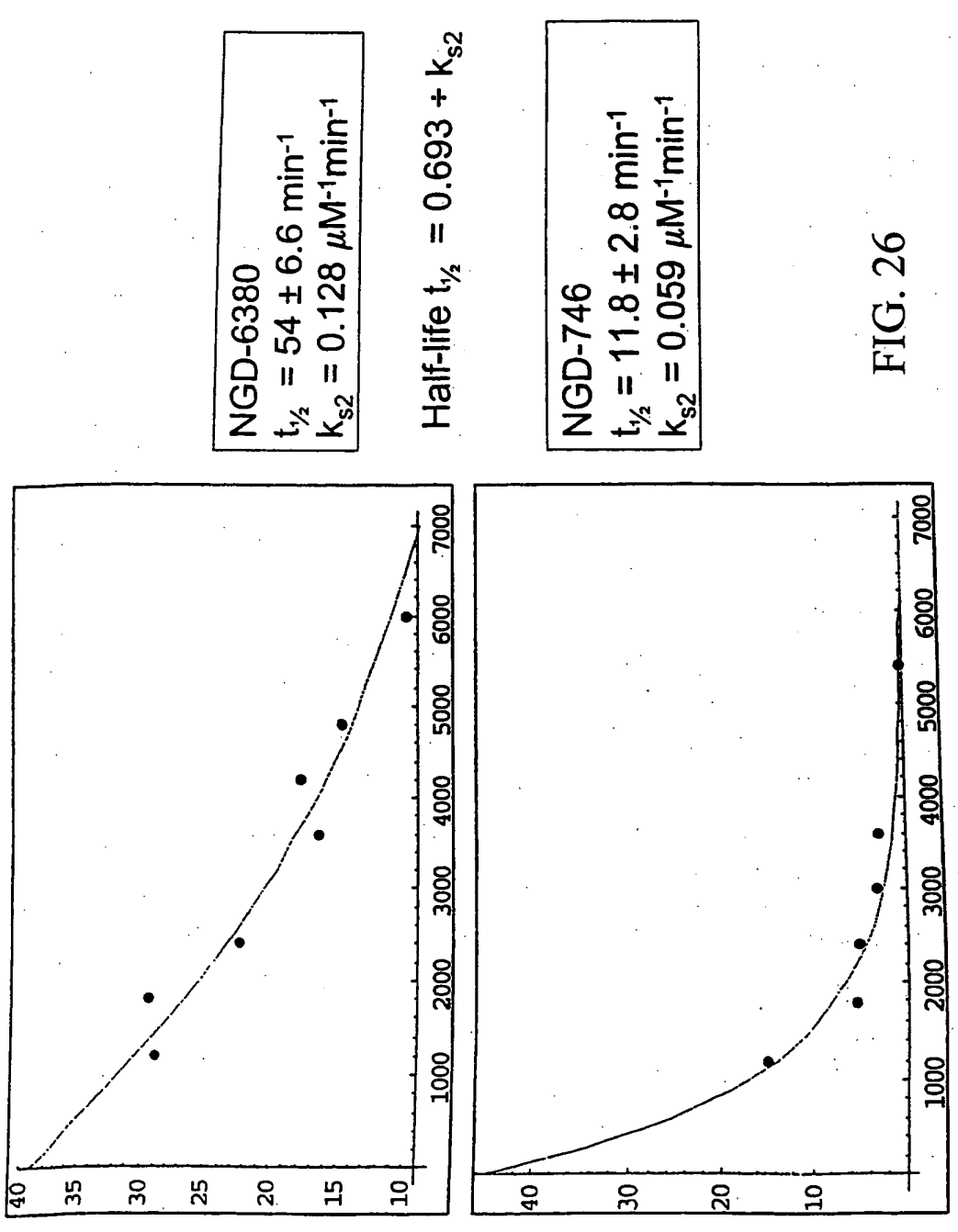
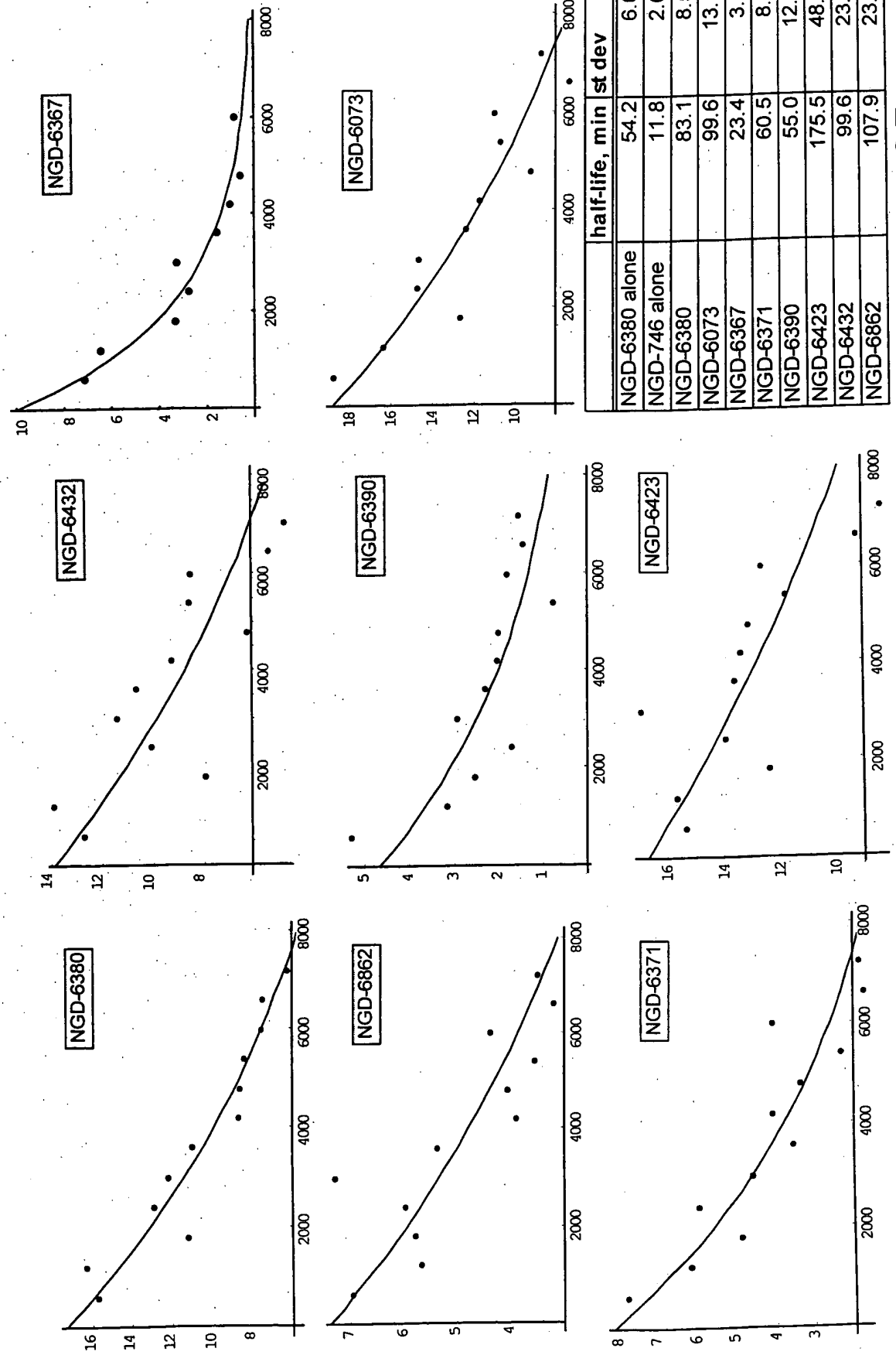

NGD-6380
$t_{1/2}$ = 54 ± 6.6 min$^{-1}$
$k_{s2}$ = 0.128 μM$^{-1}$min$^{-1}$
Half-life $t_{1/2}$ = 0.693 ÷ $k_{s2}$
NGD-746
$t_{1/2}$ = 11.8 ± 2.8 min$^{-1}$
$k_{s2}$ = 0.059 μM$^{-1}$min$^{-1}$
40
35
30
25
20
15
10
1000
2000
3000
4000
5000
6000
7000

FIG. 26




| | half-life, min | st dev |
|---|---|---|
| NGD-6380 alone | 54.2 | 6.6 |
| NGD-746 alone | 11.8 | 2.6 |
| NGD-6380 | 83.1 | 8.5 |
| NGD-6073 | 99.6 | 13.5 |
| NGD-6367 | 23.4 | 3.0 |
| NGD-6371 | 60.5 | 8.6 |
| NGD-6390 | 55.0 | 12.4 |
| NGD-6423 | 175.5 | 48.0 |
| NGD-6432 | 99.6 | 23.5 |
| NGD-6862 | 107.9 | 23.0 |

FIG. 27

| | ACE50, µM | Kd, nM |
|---|---|---|
| NGD-6380 | 3.27 | 27 |
| NGD-6862 | n/d | |
| NGD-6371 | 3.03 | 30 |
| NGD-6432 | 1.93 | 53 |
| NGD-6390 | 3.04 | 29 |
| NGD-6423 | 2.69 | 34 |
| NGD-6367 | 2.44 | 39 |
| NGD-6073 | 1.93 | 52 |
| NGD-746 | 0.58 | 500 |

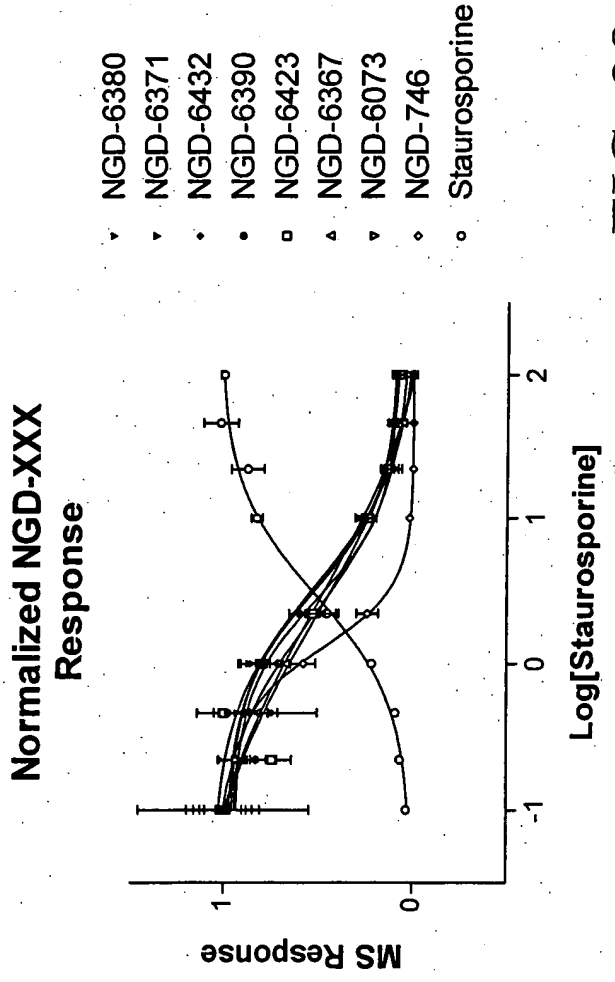



FIG. 28